# Prevalence and clinical significance of SEN-H virus in chronic hepatitis B, C and delta infections in Turkey

## Türkiye'de kronik B, C ve D hepatit infeksiyonlarında SEN-H virüsünün klinik önemi ve sıklığı

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***Background/aims:*** *SEN viruses are transmitted parenterally and can cause post-transfusion hepatitis. The prevalence and clinical significance of SEN viruses have been investigated in patients with chronic hepatitis C and B but not in D. We aimed to determine the prevalence and clinical significance of SEN viruses-H in patients with chronic hepatitis C, B and delta in Turkey.* ***Methods:*** *SEN viruses-H was analyzed in 85 patients with chronic viral hepatitis (30 HCV, 30 HBV and 25 HDV) and 43 non-professional blood donors. HBV DNA, HCV RNA and HDV RNA were positive in patients with hepatitis B, C and D, respectively. SEN viruses-H DNA was detected by semi-nested polymerase chain reaction method (L2AS, C5S primer in first step, L2AS, D11 in second step) after extraction of DNA from sera (NucleoSpin blood; Macherey-Nagel GmbH & Co KG, Germany).* ***Results:*** *SEN viruses-H DNA was found to be positive in 7/30 (23.3%), 10/30 (33.3%), 6/25 (24%), and 7/43 (16.2%) of patients with chronic C, B, and D hepatitis and healthy blood donors, respectively. There was no significant difference in clinical features and treatment response between SEN viruses-H-positive and -negative patients with chronic viral hepatitis.* ***Conclusions:*** *SEN viruses is more frequent in chronic hepatitis patients than in healthy blood donors. These results indicate that SEN viruses has no effect on the clinical course and treatment response of chronic viral hepatitis.*

**Key words:** SEN virus, chronic hepatitis

***Amaç:*** *SEN virus paranteral yolla bulaşır ve transfüzyon sonrası hepatite neden olabilir. Klinik önemi ve sıklığı kronik C ve B hepatitli hastalarda araştırılmıştır, ancak kronik D hepatitinde önemi bilinmemektedir. Bu çalışmada amaç Türkiye'de kronik C, B ve delta hepatitinde SEN virus-H sıklığı ve klinik önemini belirlemektir.* ***Yöntem:*** *Seksen beş kronik viral hepatitli hasta (30 HCV, 30 HBV ve 25 HDV) ve 43 sağlıklı kan vericisinde SEN virus-H araştırılmıştır. Hepatit B, C ve D hastalarında sırasıyla HBV DNA, HCV RNA ve HDV RNA pozitif idi. SEN virus-H DNA'sı serumdan DNA ekstraksiyonu yapıldıktan sonra (NucleoSpin blood; Macherey-Nagel GmbH & Co KG, Germany) seminested polimeraz zincir reaksiyonu ile (L2AS, C5S primer in first step, L2AS, D11 in second step) araştırılmıştır.* ***Bulgular:*** *SEN virus-H DNA pozitifliği, kronik C, B, D hepatitinde ve sağlıklı kan vericilerinde sırasıyla 7/30(23.3%), 10/30 (33.3%), 6/25(24%), 7/43(16.2%) olarak saptandı. Kronik viral hepatitli hastalarda, SEN virus-H pozitif ve negatif hastalar arasında klinik özellikler ve tedaviye cevap açısından farklılık saptanmadı.*
***Sonuç:*** *SEN virus kronik viral hepatitli hastalarda sağlıklı kan vericilerinden daha fazladır. Bu sonuçlar, SEN virusun kronik viral hepatitde tedavi cevabı ve klinik seyirde etkili olmadığını göstermektedir.*

**Anahtar kelimeler:** SEN virus, kronik hepatit

## INTRODUCTION

SEN virus (SENV) is a recently described blood-borne, single-stranded, non-enveloped DNA virus. The virus is sub-grouped into eight genotypes (A to H). However, SENV-H and D are the two genotypes thought to be associated with non-A, non-E hepatitis, and most investigations have focused on these two genotypes (1). Its prevalence is 1.8% in blood donors in the United States (1). In Japan, its prevalence is higher (10%) (2). It occurs more often in Asian people (41%, 67%, 15% in patients with hepatitis B, C and healthy adults, respectively) (3). SENV is transmitted parenterally and can cause post-transfusion hepatitis (4). Infection is cleared spontaneously in the majority of patients; approximately 45% develop a persistent infection that exceeds one year and has been documented as long as 12 years (5). The prevalence and clinical significance of SENV have been inves-

tigated in patients with chronic hepatitis C and hepatitis B but not in D hepatitis. Coinfection with hepatitis B virus (HBV), hepatitis D virus (HDV) or hepatitis C virus (HCV) has been reported to be associated with severe and progressive liver disease (6). However, the clinical importance of SENV infection alone or in combination with the other hepatotropic viruses remains controversial. In this study, we aimed to determine the prevalence and clinical significance of SENV-H in patients with chronic hepatitis C, B and D in Turkey.

## MATERIALS AND METHODS

SENV-H was retrospectively analyzed in serum samples of 85 patients with chronic viral hepatitis (30 HCV, 30 HBV and 25 HDV) and 43 non-professional blood donors. In 14 chronic hepatitis B patients and 22 delta patients, hepatitis B was "e" antigen (HBeAg)- negative. Diagnosis of chronic viral hepatitis was based on clinical signs, laboratory findings and liver biopsy characterizations. Liver biopsy was performed in all patients. Chronic hepatitis was defined and graded by Knodell scoring system (7). HCV RNA for HCV, HBV DNA for HBV and HDV RNA for HDV infection were positive in all patients.

In patients with chronic hepatitis B, lamivudine (100 mg/day, per os [po], 52 weeks) or lamivudine plus interferon (IFN) alpha 2a (9 million U, subcutaneously [sc], 3 times weekly, 52 weeks); in patients with hepatitis C, IFN alpha 2a (9 million U, sc, 3 times weekly, 48 weeks) plus ribavirin (1000-1200 mg/day, po, 48 weeks); and in those with HDV, IFN alpha 2b (10 million U, sc, 3 times weekly, 24 months) plus ribavirin (1000-1200 mg/day, po, 24 months) treatments were given.

Antibody to hepatitis C virus (anti HCV) was tested by third generation UBI HCV EIA 4.0 kit (Organon Technika, RM Boxtel, Netherlands) and anti-HIV by Vironostika HIV Uni-Form II plus O kit (Organon Technika, RM Boxtel, Netherlands) that is based on a technique called "sandwich enzyme immunoassay". HCV RNA was determined by polymerase chain reaction (PCR) (Cobas Amplicor HCV kit, Version 2.0, Roche Molecular System Inc., Branchburg, USA). Hepatitis B surface antigen (HBsAg), HBeAg, antibody to hepatitis B "e" antigen (anti HBe) (Sanofi Pasteur Diagnostics, USA) and anti-HDV (Abbott Laboratories, IL, USA) were determined by using immunoenzymatic assays. HBV DNA was quantitated by hybridization technique (Digene hybrid capture system,

Digene Corp., USA). Lower detection limit of this assay was 4 pg/ml.

For the diagnostic HDV RNA PCR and genotyping PCR, HDV RNA was extracted by Boom extraction method (8). 100 µl serum sample was used appropriately for protocol. Then complementary DNA (cDNA) was purified by using 10 µl extracted material, 200 ng random primer (Roche; catalog no.1 034 731; Roche Diagnostics GmbH, Mannheim, Germany), 0.4 mM dNTP, 20 U RNAse inhibitor (Sigma; R2520; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 5 U AMV (Avian myeloblastosis virus) reverse transcriptase (Roche; catalog no. 1 495 062; Roche Diagnostics GmbH, Mannheim, Germany) and 1X buffer (in total 25 µl reverse transcription volume). cDNA mixture was incubated at 42°C for 1 h and 95°C 5 min.

HDV RNA was detected for diagnosis of patients by RT nested PCR (9). In the first step of PCR, 5 µl cDNA was added to the mixture of PCR containing 10 mM Tris HCl, 1.5 mM MgCl2, 50 mM KCl, 0.2 mM dNTP, 0.05 mM concentration of outer primers 5413 (5' – GCC CAG GTC GGA CCG CGA GGA GGT) and 8276 (5'- ACA AGG AGA GGC AGG ATC ACC GAC), and 2.5 U Taq DNA polymerase enzyme (Sigma; D1806; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (total volume 50 µl). The reaction mixture was incubated at 94°C 1 min, 55°C 1 min, and 72°C 1 min for 35 cycles. After the incubation at 72°C for 10 min, the process was finalized. The same PCR cycle conditions and reagent concentrations were used in the second PCR step with inner primers 5414 (5'-GAG ATG CCA TGC CGA CCC GAA GAG) and 5415 (5' GAA GGA AGG CCC TCG AGA ACA AGA) and 5 µl first PCR product. Results of PCR were analyzed by electrophoresis in 1.5% agarose gels. The genotype analyses were determined by RT-PCR and RFLP (restriction fragment length polymorphism) methods (10). For PCR step, 10 µl cDNA was added to the mixture of PCR that includes 10 mM Tris HCl, 1.5 mM MgCl2, 50 mM KCl, 0.2 mM dNTP, 0.05 mM concentration of primers 900s (5'-GCC GAC CCG AAG AGG AAA G) and 1280as (5'-GAA GGA AGG CCC TSG AGA ACA AGA), 2.5 U Taq DNA polymerase enzyme (Sigma; D1806) (total volume 50 µl). The reaction mixture was incubated at 94°C for 9 min and then for 40 cycles of 94°C 45 s, 58°C 30 s, and 72°C 45 s. After incubation at 72°C for 10 min, the process was finalized. Results of PCR were analyzed by electrophoresis in 1.5% agarose gels. For HDV genotyping with

RFLP analysis, digestion was performed in 5 µl samples with 5 U SmaI enzymes (Fermentas ER0662) and 1X buffer (total 20 µl volume), and then the mixture was incubated overnight. Digested products were analyzed by electrophoresis in 2% agarose gels.

The HCV genotype was also tested by line probe assay (INNO-LiPA HCV II Innogenetics N.V., Cohent, Belgium).

The presence of SENV-H DNA was determined by semi-nested PCR by using primer as described previously with two base modifications in the second PCR sense primer (1, 11). DNA extraction was performed from 200 µl of serum using nucleic acid extraction kit NucleoSpin Blood (Macherey-Nagel GmbH & Co KG, Germany). For the first PCR, 50 µl of reaction mixture containing 10 µl DNA sample, 10 mM Tris HCl, 1.5 mM MgCl2, 50 mM KCl, 0.2 mM dNTP, 0.05 mM concentration of outer primers (11) L2as (5' –CCT CGG TTK SAA AKG TYT GAT AGT) (K= G or T, S= C or G, Y= C or T) and C5s (5'-GGT GCC CCT WGT YAG TTG GCG GTT ) (W= A or T), 2.5 unit Taq DNA polymerase enzyme (Biotools cat. no. 10 047; Biotools B&M Labs S.A., Madrid) was amplified. The reactions consisted of preheating at 94°C for 2 min, 35 cycles of denaturizing at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final incubations at 55°C for 5 min and 72°C for 5 min. The second PCR step was carried out with 50 µl PCR reaction mixture containing the same reagent concentrations used for the first PCR step, 5 µl of first amplification product, antisense primer L2AS and sense primer from Schröter et al. (11) with two base modifications D11 (5' – TCS GGT CTG CTC ACC ACW ACC). The same PCR cycle conditions were used in the second PCR step. Amplification products were separated by 3% agarose gel electrophoresis stained with ethidium bromide and examined under UV light (Figure 1).
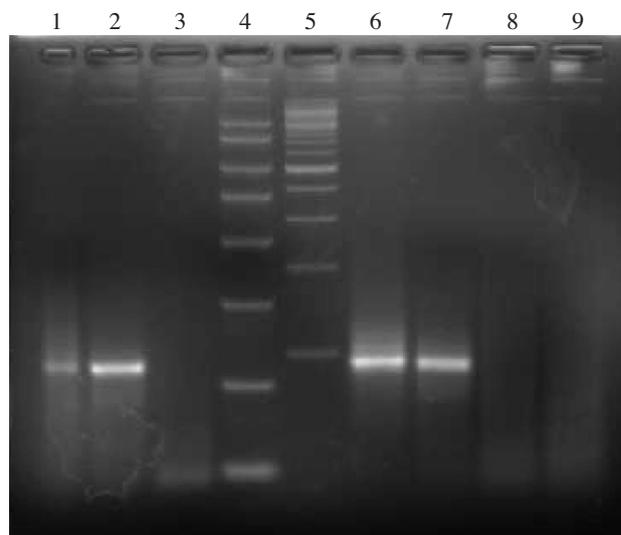


**Figure 1.** Results of agarose gel electrophoresis: 1, 2, 6, 7 SENV-H positive (188 bp); 3, 8, 9 SENV-H negative; 4 gel marker 1 (50, 150, 300, 500, 750, 1000, 1500, 2000 bp), gel marker 2 (200, 400 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 3000 bp).

For statistical analyses, nonparametric tests in the SPSS program (SPSS for Windows, release 13, SPSS, Chicago, IL, USA) were applied; p values less than 0.05 were considered to be significant.

## RESULTS

One hundred and twenty-eight cases (mean age: 43±28 years) were evaluated for SENV-H positivity. Of the patients, 85 were chronic viral hepatitis and 43 blood donors. Gender, serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and albumin levels were similar between groups, whereas mean age and serum gamma globulin levels were higher in the HDV group and patients with chronic hepatitis B had higher serum ALT levels (Table 1). More advanced stage was observed in liver biopsies of the HDV group (Table 1).

**Table 1.** Biochemical, demographic and histological features of patients

|  | HCV | HBV | HDV | P* |
|---|---|---|---|---|
| Gender (F/M) | 11/19 | 9/21 | 5/20 | >0.05 |
| Age, years | 43.6±13 | 32±13 | 51.6±68.6 | <0.05 |
| AST, IU/L | 73.4±28.4 | 79.3±36.6 | 66.5±38.2 | >0.05 |
| ALT, IU/L | 119.1±54.3 | 149.4±97 | 102±54.7 | <0.05 |
| ALP, IU/L | 216.7±97 | 198.3±93.2 | 214.5±97 | >0.05 |
| GGT, IU/L | 71.7±77 | 38.9±21.7 | 67.4±51 | >0.05 |
| Albumin, g/dl | 4.2±0.4 | 4.1±0.3 | 4±0.4 | >0.05 |
| Gamma globulin, g/dl | 1.8±0.7 | 1.4±0.2 | 2.3±0.5 | <0.05 |
| HAI (mean) | 6.3±3.1 | 6.2±2.1 | 8.6±3.8 | >0.05 |
| Stage 1/2/3/4 (n) | 14/9/6/1 | 17/8/2/3 | 4/6/5/10 | <0.05 |

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. ALP: Alkaline phosphatase. GGT: Gamma glutamyl transpeptidase. HAI: Histological activity index. * Kruskal-Wallis test.

Genotypic analyses revealed genotype I (DNA patterns 227 bp and 178 bp) in all HDV, genotype D in all HBV and genotype 1b in 90% of HCV patients.

Eleven patients with chronic hepatitis B were treated with lamivudine (100 mg/day, po, 52 weeks) and 19 with lamivudine plus IFN alpha 2a (9 million U, sc, 3 times weekly, 52 weeks). End of follow-up response rate (EFR) was 55%. In SENV-positive patients, EFR rate was 30%, and this was not statistically significant when compared with the total group and with the SENV-negative group (53.8%).

In patients with HCV, IFN alpha 2a (9 million U, sc, 3 times weekly, 48 weeks) plus ribavirin (1000-1200 mg/day, po, 48 weeks) therapy was administered and a EFR of 70% was obtained. EFR rates of SENV-positive and -negative patients were 65% and 42.9%, respectively (p>0.05). Treatment was discontinued in those with positive HCV-RNA at month 6.

Twenty-five patients with HDV were evaluated; six of them were followed up without therapy (advanced age and stage). Nineteen of them were given IFN alpha 2b (10 million U, sc, 3 times weekly, 24 months) plus ribavirin (1000-1200 mg/day, po, 24 months). EFR rate was 42.1%. In SENV-positive and -negative patients, EFR rates were 50% and 23.5%, respectively (p>0.05). These patients were included from a published trial of our center (12).

SENV-H DNA was found to be positive in 7/30 (23.3%), 10/30 (33.3%), 6/25 (24%), and 7/43 (16.2%) patients with chronic C, B, and delta hepatitis and healthy blood donors, respectively (Figure 2). There was no significant difference in clinical features, laboratory findings and treatment response rate between SENV-H-positive and -negative patients with chronic viral hepatitis (Table 2).

## DISCUSSION

In all of the liver disease groups, the prevalence of SENV is higher than in the healthy blood donor controls (13). However, the clinical impact of SENV infection and the relation with liver disease are still unclear. Thus, we investigated the prevalence of SENV infection in patients with chronic hepatitis and compared with healthy donors. Although SENV is a highly prevalent infection in Japan (20%) and has been discovered as part of a se-
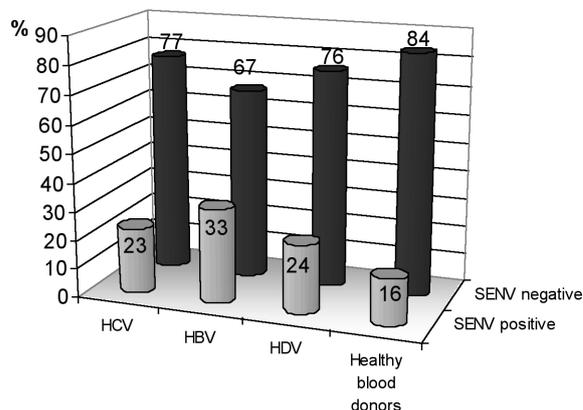


**Figure 2.** SENV-H positivity rates in all cases.

arch for causes of post-transfusion hepatitis, there is no firm evidence that SENV infection either causes hepatitis or worsens the course of coexistent liver disease (4). SENV was positive in 31% of healthy Chinese people, and in 36% (74/126) of chronic hepatitis B and 85% (17/20) of chronic hepatitis C patients (14). In Taiwan, SENV-H prevalence was reported as 30.5% in blood donors (15). On the other hand, its prevalence is lower in America and Italy (2-3%) (1, 16). Consistent with the geographical localization, our results from Turkey were between these rates. Although not statistically significant, SENV was more prevalent in patients with chronic hepatitis (33% in HBV, 24% HDV and 23% HCV) than in healthy individuals (16.6%) in our study.

This is the first study to show SENV prevalence in delta hepatitis (triple infection). The frequency of SENV appears to be higher in patients with hepatitis B than with hepatitis C. In delta hepatitis, se-

**Table 2.** Biochemical, demographic and histologic features of SENV-positive and -negative chronic hepatitis patients

| n | SENV-negative (62) | SENV-positive (23) | P* |
|---|---|---|---|
| **Gender (F/M)** | 23/44 | 2/16 | >0.05 |
| **Age, years** | 43.6±43 | 35±13 | >0.05 |
| **AST, IU/L** | 72±35 | 80±31 | >0.05 |
| **ALT, IU/L** | 116±64 | 153±99 | >0.05 |
| **ALP, IU/L** | 207±94 | 217±97 | >0.05 |
| **GGT, IU/L** | 58±60 | 64±42 | >0.05 |
| **Albumin, g/dl** | 4±0.3 | 4.2±0.3 | >0.05 |
| **Gamma globulin, g/dl** | 1.9±0.7 | 1.6±0.3 | >0.05 |
| **HAI (mean)** | 7.3±3.3 | 6.3±2.6 | >0.05 |
| **Stage 1/2/3/4 (n)** | 25/16/12/13 | 10/7/1/1 | <0.05 |

*Wilcoxon test

rum gamma globulin levels were higher than in the other patients; this may be related to older age and advanced stage of these patients. The relation with SENV was not considered. We also evaluated treatment response of the patients, but there was no difference between SENV-positive and -negative patients. Umemura et al. (5) evaluated the effects of SENV in chronic hepatitis C treated with IFN alpha; they showed that SENV infection had no apparent influence on the severity of HCV-related liver disease or the HCV response to IFN-alpha.

Here we first report the association of SENV, HBV and delta virus. Triple or dual infection with SENV does not affect the severity of liver disease or treatment response rate.

In conclusion, SENV-H is a frequent phenomenon in chronic hepatitis compared with healthy blood donors. Its frequency is similar in patients with HBV, HCV and HDV. It has no effect on clinical features, biochemical parameters or therapy response.

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