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Prevalence of SEN virus genotype-D and genotype-H among haemodialysed patients

Aim: A recently discovered DNA virus (SEN virus) has been assumed to be responsible for post-transfusion hepatitis in humans. Phylogenetic analysis of the SEN virus (SEN-V) has revealed the existence of 8 different genotypes (A-H). SEN-V genotype-H (SENV-H) and SEN-V genotype-D (SENV-D) have been described as most closely associated with post-transfusion hepatitis. So far, it is unclear whether patients on maintenance haemodialysis are at increased risk for acquiring the SEN virus. The aim of the present study was to investigate the prevalence of SENV-D and SENV-H among patients on maintenance haemodialysis.

Materials and Methods: Serum samples derived from 100 haemodialysed patients were examined for SENV-D and SENV-H viraemia by polymerase chain reaction (PCR). One hundred and twenty serum samples were obtained from healthy blood donors, who served as the control group.

Results: The prevalence of SENV-D was 33% (n = 33) while that of SENV-H was 22% (n = 22) among the patients on maintenance haemodialysis. The prevalence of SENV-D was 5% (n = 6) while that of SENV-H was 20% (n = 24) among the healthy blood donors. Our data suggest that SEN-V infection was significantly more prevalent (P < 0.05) in patients on haemodialysis (55%) than in control subjects (25%).

Conclusions: These findings reveal that patients on maintenance haemodialysis are at risk of SEN-V infection. Another important finding is the relatively high prevalence (25%) of SEN-V in healthy blood donors in our region.

Key Words: SEN-V, SENV-D, SENV-H, haemodialysis, blood donor, PCR

Hemodiyaliz hastalarında SEN virus genotip-D ve SEN virus genotip-H prevalansı

Amaç: Yakın zamanlarda keşfedilen bir DNA virusu olan SEN virus (SEN-V) insanlarda post transfüzyon hepatitinden sorumlu tutulmaktadır. SEN-V genomunun filogenetik analizi, bu virusun 8 farklı genotipinin (A-H) varlığını ortaya koymaktadır. Bu genotiplerden iki tanesi; SEN virus genotip D (SENV-D) ve SEN virus genotip H (SENV-H)'nin post transfüzyon hepatitleri ile yakın ilişkili olduğu belirtilmektedir. Şimdiye kadar yapılan çalışmalar ile hemodiyaliz yapılan hastaların, SEN-V'un bulaşmasında artan bir riske sahip olup olmadıkları netlik kazanmamıştır. Bu çalışmadaki amacımız, hemodiyaliz ile tedavi edilen hastalarda SENV-D ve SENV-H'nin prevalansını araştırmaktır.

Yöntem ve Gereç: Hemodiyaliz hastalarından elde edilen 100 serum örneği, SENV-D ve SENV-H yönünden polimeraz zincir reaksiyonu (PZR) ile incelendi. Sağlıklı kan donörlerinden elde edilen 120 serum örneği kontrol grubu olarak kullanıldı.

Bulgular: Hemodiyaliz ile tedavi edilen hastalarda SENV-D prevalansı % 33 (n = 33), SENV-H prevalansı % 22 (n = 22) olarak saptandı. Kan donörlerinde ise SENV-D prevalansı % 5 (n = 6), SENV-H prevalansı % 20 (n = 24) olarak saptandı.

Sonuç: Bizim verilerimiz SEN-V infeksiyonunun hemodiyaliz ile tedavi edilen hastalarda (% 55), kontrol grubundan (% 25) daha yaygın (P < 0,05) olduğunu ve bu grupta SEN-V infeksiyonunun yüksek riskini göstermektedir. Diğer önemli bulgu ise, bölgemizdeki sağlıklı kan donörlerinde SEN-V'un oldukça yüksek (% 25) prevalansıdır.

Anahtar Sözcükler: SEN-V, SENV-D, SENV-H, hemodiyaliz, kan donörü, PZR

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Introduction

Five hepatitis viruses (A-E) have been established for viral hepatitis cases and there are still patients with acute or chronic hepatitis with unknown origin (non-A to -E hepatitis) (1). Both the hepatitis-G virus (HGV) and the TT virus (TTV) have been indicated as candidates for new hepatitis viruses. However, previous detailed investigations regarding each of these viruses have shown that they are not a significant causative agent of transfusion associated non-A to -E hepatitis (1,2).

Recently, a new virus was discovered that might be the primary cause of non-A to -E hepatitis. This virus is named SEN, which was formed from the initials of the patient from whom it was recovered in the serum, an intravenous drug abuser infected with human immunodeficiency virus (HIV) (3,4). The SEN virus (SEN-V) is a member of the family *Circoviridae*, a group of non-enveloped, circular DNA viruses (4-6) that also include the recently identified TTV and its variants SANBAN, YONBAN, TUS01, and PMV. However, further studies revealed that this virus is distantly related to the TTV family (6).

The SEN-V genome is a single stranded DNA and approximately 3800 nucleotides in length (4,5). To date, phylogenetic analysis of SEN-V has demonstrated 8 different genotypes: SENV-A to SENV-H (2,7). SENV-D and SENV-H genotypes are related to transfusion-associated non-A to -E hepatitis (7) and are more prevalent within the population exposed to transfusion (8). Nevertheless, these genotypes have been found at various rates in different populations and the role of SEN-V regarding of the pathogenesis of liver disease is not yet known (9,10).

Active infection is frequent in healthy blood donors and in the general population. This high prevalence is only explained by some SEN-V strains; especially SENV-B, SENV-A, and SENV-E are less frequently found among blood donors and do not appear to be related to non-A to -E hepatitis (6,11). In contrast, genotypes-D and -H have only been found in 1% of blood donors but in more than 50% of non-A to -E hepatitis cases. Chronic infection is detected in patients with various hepatic diseases

(11). Despite the favourable ratio of donors/acute hepatitis for SEN-V genotype-D and -H (the fact that preliminary data suggest that SEN-V can replicate in the liver), no true association between SEN-V and liver damage has been proven so far (7). Little is known of the natural history of the infection. Chronic infections of over a decade have been observed in retrospectively tested samples of infected individuals, but most patients clear viraemia during the first months of exposure. Therefore, true exposure to the virus is difficult to assess, as no serological test for SEN-V antibodies is currently available (11).

SEN-V is transmitted by blood, as demonstrated by comparing sequence homology between donor and recipient (12). Moreover, transfused patients are at higher risk of acquiring SEN-V than non-transfused patients. Risk of infection in transfused patients increased proportionally with the number of units of blood transfused (13). However, many studies suggest that there is no association between SEN-V and liver pathology. In our recent study, SEN-V was detected at almost the same frequency in patients with high alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels but was negative for HBV-DNA and HCV-RNA and without any transfusion history and the control group. Therefore, we had also suggested that SEN-V does not seem to contribute to the pathogenesis of liver disease (P > 0.05) (14). However, it is still unclear whether these viruses are orphans.

Patients on maintenance haemodialysis are considered to be at risk of infection by blood-borne viruses, because the medical treatment processes are frequently associated with intravenous drug injection and blood transfusions (1). In this study, we aimed to investigate the presence of SENV-D and SENV-H genotypes in order to determine the prevalence of SEN-V infection in patients on maintenance haemodialysis and in healthy blood donors in the city of Mersin, Turkey.

Materials and Methods

Serum samples and study populations

The serum samples were obtained from 100 patients on maintenance haemodialysis considered

to be at risk of blood-borne infections at a private haemodialysis centre in Mersin in 2004. One hundred and twenty serum samples were also obtained from healthy volunteer blood donors as a control group without a blood-borne contact history and negative for routine donor screening tests at the blood bank department of Mersin University hospital. All serum samples were stored frozen at –80 °C until analysis.

Viral DNA isolation

Viral DNA isolation was performed by extraction of nucleic acid from serum samples. We used a modified and optimized phenol-chloroform and chloroform DNA extraction protocol from a previously published procedure (15). Briefly, 100 μl serum samples were mixed with 300 μl of lysis buffer (13.3 mmol/l Tris-HCl [pH 8.0], 6.7 μmol/μl ethylene-diamine-tetra-acetic acid, 0.67% sodium dodecyl sulphate, and 133 mg/ml proteinase-K) and incubated at 56 °C for 4 h. Two phenol-chloroform extractions were followed by 1 chloroform extraction, and DNA was precipitated with ethanol. The DNA was eluted in 25 μl of DNase/RNase free water. This was stored frozen at –20 °C until analysis and used as a template for amplification.

Detection of SENV-D and SENV-H genotypes

Analysis of SENV-D and SENV-H genotypes was performed by polymerase chain reaction (PCR) with type-specific primers according to research articles by Umemura et al. and Kojima et al. (7,16) with several modifications. Primers D10S and L2AS and primers C5S and L2AS were used for SENV-D and SENV-H detections, respectively (Table 1). SENV-D and SENV-H genotypes were analysed using the same PCR conditions.

PCR reactions for amplification of each sample were carried out in a 50 μl PCR mixture containing PCR buffer, 0.25 μmol/μl of nucleotide mix (Promega, Madison, WI, USA), 0.2 μM of each primer, 2.5 μmol/μl magnesium chloride, 1.75 U *Taq* polymerase (Promega), and 10 μl of sample DNA as template. The sample was amplified in a thermal cycler (Eppendorf, Mastercycler, Germany). The amplification protocol consisted of 5 min of pre-denaturing at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min 72 °C, and then by a final extension at 70 °C for 10 min. The products (222 base pairs [bp] for SENV-D and 229 bp for SENV-H) of PCR were separated using 2% agarose gel, stained with ethidium bromide, and visualised under a UV illuminator.

Statistical analysis

Differences between the groups were examined by the 2 proportion comparison method using MINTAB 13.0 software. P values < 0.05 were considered significant.

Results

SENV-D was detected in 33 of the 100 (33%) patients by genotype-specific PCR with genotype D-specific primers. With the genotype H-specific primers, SENV-H was detected in 22 (22%) of the 100 patients. In the control group, SENV-D was detected in 6 (5%) and SENV-H was detected in 24 (20%) of the 120 blood donors (Table 2). The genotypes were determined by size comparison of the PCR products with DNA marker (Figure).

There were also significant differences for SEN-V cases between the patient and control groups.

Table 1. Primers used to detect SEN-V.*

Sense primer (Sequence)	Antisense primer (Sequence)	Region Amplified (nt.)
D10S (5'-GTAACTTTGCGGTCAACTGCC-3')	L2AS(5'-CCTCGGTTKSAAAKGTYTGATAGT-3')	1322-1544[a]
C5S (5'-GGTGCCCCTWGTYAGTTGGCGGTT-3')	L2AS(5'-CCTCGGTTKSAAAKGTYTGATAGT-3')	1271-1500[b]

W: A or T, Y: C or T, M: A or C, K: G or T, S: C or G

[a] Obtained from the sequence of SENV-D (AX025730)

[b] Obtained from the sequence of SENV-H (AX025838)

* These data were taken from the research article by Kojima et al. (16)

Table 2. Prevalence of SEN-V infections in patient and control groups.

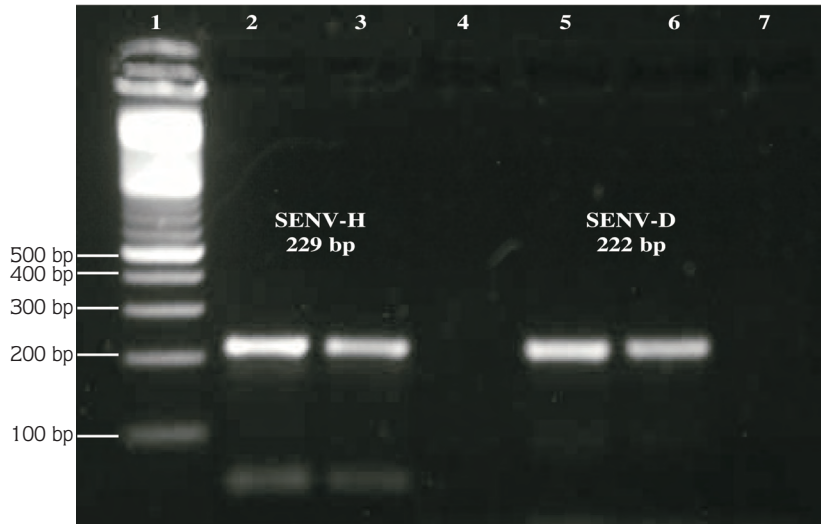| Study groups	Total (no.)	SEN-V positive no. (%)	SENV-D positive no. (%)	SENV-H positive no. (%)
Patient group	100	55 (55)	33 (33)	22 (22)
Control group	120	30 (25)	6 (5)	24 (20)

Figure.   PCR products of SENV-D and SENV-H on 2% agarose gel. Lane 1; 100 bp DNA step ladder (Promega), lane 2 and 3; 229 bp PCR product of SENV-H DNA, lane 4; SENV-H DNA negative sera sample, lane 5 and 6; 222 bp PCR product of SENV-D DNA, lane 7; SENV-D DNA negative sera sample.

Although there were significant differences for SENV-D cases ($P < 0.05$), there were no significant differences for SENV-H cases ($P > 0.05$) between the patient and control groups.

Discussion

Initial studies of SEN-V genotypes were organised by Dr. Primi in Diasorin Laboratory, Italy. The prevalence of 5 SEN-V genotypes (A, B, H, D, and E) and consensus sequence designed from total SEN-V were measured in various patients and donor populations. These studies demonstrated that SENV-D and SENV-H were found in high prevalences in transfusion associated non-A to -E hepatitis. Therefore, systematic research on SENV-D and SENV-H in transfused populations and healthy blood donors has intensified (7). We

performed this study in order to investigate the prevalence of SENV-D and SENV-H in serum of patients on maintenance haemodialysis and healthy donors as controls in Mersin.

The prevalence of SENV-D/H DNA was 55% (SENV-D: 33% and SENV-H: 2%) and 25% (SENV-D: 5% and SENV-H: 20%) among haemodialysed patients and healthy blood donors, respectively. SENV-D/H infection was found more frequently in haemodialysed patients than in healthy blood donors. We suggest that the high prevalence in this group was probably associated with long-term intravenous drug injection with contaminated devices and contamination with infected blood through the chambers of the haemodialysis instruments. This higher frequency in the patient group implies that great attention should be paid to SEN-V transmission risk during haemodialysis as is

paid to other blood-borne viruses such as HBV, HCV, and HIV. It was reported that haemodialysed patients are considered to be at risk of blood-borne infection and previous studies indicated a high prevalence of transfusion-transmitted viral agents such as HCV, HGV, and TTV (17,18).

The prevalence of SENV-D/H was detected as 38% by Kobayashi et al., in Japan, as 68% by Kao et al., in northern Taiwan, and as 61.6% by Dai et al., in southern Taiwan, among haemodialysed patients (1,19,20). The prevalence of SENV-H was reported as 12.8% among maintenance haemodialysis in Germany and as 16.8% among healthy blood donors by Schröter et al. Because of no significant differences for SENV-H cases between patient and control groups, they reported that it was not necessary to dialyse SENV-H viraemic patients on separate machines (21). Our results also demonstrated that the rate of SENV-H viraemia was similar among patient (22%) and control groups (20%) in our region. In any event, Schröter et al. previously suggested that SENV-H might be establishing a commensal relationship with its host, resembling TTV in SENV-H viraemic individuals (22). Therefore, both groups of our samples could also be examined for TTV.

In Turkey, the prevalence of SENV-D and SENV-H in patients on maintenance haemodialysis was previously reported as 10.1% and as 16.8%, respectively by Toraman et al., in Elazığ province (23). According to our data, the prevalence was not similar to that in different regions of Turkey. However, the higher rate of SEN-V in our results indicates a similarity with Taiwanese haemodialysed patients (19,20). These results indicate that SEN-V has a different geographic distribution and is fairly common around the world.

Another interesting finding is the relatively high prevalence (25%) of SENV-D/H viraemia (SENV-D: 5% and SENV-H: 20%) in healthy blood donors, which implies that it is widespread among the general population in our region. On the other hand, many other studies report that the frequency of SENV-D/H is high among healthy individuals; SENV-D/H prevalence was reported as 15% (19), 24.2% (24), and 51% (25) by various researchers in

Taiwan. Moreover, SENV-D/H prevalence was detected as 10% (26) and 28.6% (27) in Japan, 2-3% in Italy and America (7), and 39% (28) in Canada. Our results are similar to the findings reported by Dai et al. in Taiwan (24.2%) (24) but higher than those reported by Shibata et al. in Japan (10%) (26), by Kao et al. in Taiwan (15%) (19), and in Italy and America (2-3%) (7). Clearly its prevalence have been found to vary in different populations and geographic regions. Therefore, this virus is seen as endemic in the exposed area. These findings suggest that this virus is probably transmitted by other parenteral routes. Indeed, recent data suggest that SENV-D/H could be transmitted by both parenteral and non-parenteral routes and that its transmission pattern might differ from that of HCV and HGV, but is similar to that of TTV (10).

The rate of SENV-H viraemia in our study was also higher than the findings reported by Schröter et al. (21) and Toraman et al. (23). However, this could be related to the sensitivity and specificity of the primers we used. We have chosen the primers from the research articles by Umemura et al. and Kojima et al. (7,16). Kojima et al. (16) have reported the sensitivity and specificity of the genotype specific primers to be both 100% for SENV-D. However, while the sensitivity was 100%, the specificity was 64% for SENV-H. These findings suggest that confirmation by hybridization or sequencing could be done for genotype-H. Therefore, our results for genotype-H might need confirmation.

It was reported that the prevalence of SENV-D/H infection after transfusion was 30%, while the prevalence of SENV-D and SENV-H was 32.7% and 37.5%, respectively, in the United States (7,29). Another study also reported that the prevalence of SENV-D and SENV-H viraemia was 10.3% and 35.6% in a transfused population, respectively, in the United States (3). SENV-D/H prevalence was reported as 44.4% in Germany (22). All of these data suggest that SEN-V transmission via blood could be important during transfusion from infected donors. However, there is no routine donor screening test performed for SEN-V in the blood bank. Although SEN-V has not been associated with liver disease, it might be quite important if this novel virus is

associated with a pathology other than liver disease in the future.

In conclusion, many healthy people are infected with SEN-V in our region. Probably, primary exposure occurs with this virus not only via the parenteral route, but also by some other routes such as the faecal-oral route in our region. Further studies are still required in order to determine the transmission routes of this novel virus. SEN-V infection among haemodialysed patients at risk

group for transfusion-transmitted disease is significantly higher than among healthy blood donors. These findings indicate that more attention should be paid during haemodialysis.

Acknowledgements

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