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Full Length Research Paper

Epidemiological Study of SEN Virus Infection Among Blood Donors and Chronic Hepatitis B and C Patients in Chaharmahal Va Bakhtiari Province

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***SEN* virus (*SEN-V*) is a blood-borne, circular, nonenveloped and single-stranded DNA virus. Phylogenetic analysis demonstrated 9 different genotypes for this virus. *SEN-V* could be related to post-transfusion hepatitis and infections with this virus in blood donors and hepatitis patients differ markedly by geographic region. The purpose of present study was to determine the prevalence of H and D genotypes of *SEN-V* (*SENV-H* and *SENV-D*) infection in blood donors and patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) for the first time in Chaharmahal Va Bakhtiari province located in southwest Iran. *SEN-V* DNA was analyzed in 240 serum samples of the patients with chronic HBV and HCV (172 HBV and 68 HCV) and 60 non-professional blood donors from the blood transfusion organization, hospital and clinical and pathological laboratories in Chaharmahal Va Bakhtiari province. *SEN-V* DNA was amplified by specific primers for *SENV-H* and *SENV-D* genotypes using polymerase chain reaction (PCR) method after extraction of DNA from sera and PCR products were visualized in a 1% agarose gel electrophoresis. *SENV-H* genotype was found to be positive in 54/172 (31.39%), 23/68 (33.82%), and 8/60 (13.33%) and *SENV-D* genotype was detected in 48/172 (27.91%), 27/68 (39.7%), and 6/60 (10%) of patients with chronic HBV, HCV and healthy blood donors, respectively. These results showed that high prevalence of *SEN-V* infection in patients with chronic HBV and HCV compared healthy blood donors in Chaharmahal Va Bakhtiari province using T test statistical analysis (P<0.05). According to these findings examination of serum samples for control and prevention of *SEN-V* infection in hepatitis patients and healthy blood donors seems to be necessary**.

Key words: *SEN* virus, hepatitis B virus (HBV), hepatitis C virus (HCV), polymerase chain reaction (PCR), Chaharmahal Va Bakhtiari province.

INTRODUCTION

SEN virus (*SEN-V*) is a member of the Circoviridae family, a group of small, circular DNA virus that includes *TT* virus (*TTV*), *TUS01*, *SANBAN*, and *YONBAN* (Sharifi et al., 2008). This virus is blood-borne and nonenveloped with approximately 3800 nucleotides in length and about 26 nm in size and in these viruses at least three open reading frames (ORF) have been identified (Dai et al., 2006). The ORF1 with Arg/Lys-rich domains is the largest ORF with hydrophilic characteristic (Mikuni et al., 2002). The role of ORF2 is yet to be determined. The ORF3 translation results in the formation of a protein with a homology amid a DNA topoisomerase I, therefore ORF3 seems to play a significant role in the replication of the virus (Sagir et al., 2004). Interestingly, *SEN-V* have high mutation rate (7.32*10-4 per site per year) and it is more similar to RNA viruses rather than DNA viruses and it could be the ability of persistence of *SEN-V* in the host cells (Umemura et al., 2002).

Viral hepatitis (A to E) is still one of the important agents of acute and chronic liver disease worldwide (Moriondo et al., 2007). *SEN-V* genotype H and D (*SENV-H*, *SENV-D*) can cause post-transfusion hepatitis

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Abbreviations: HBV, Hepatitis B virus; **HCV,** hepatitis C virus, **PCR,** polymerase chain reaction.

and infections with this virus in blood donors and hepatitis patients differ markedly by geographic region. *SEN-V* is transmitted by blood, as demonstrated by comparing the sequence homology between donors and recipients (Shibata et al., 2001). However, further studies revealed that this virus is distantly related to the *TTV* family (Davidson et al., 1999). To date, phylogenetic analysis of *SEN-V* has demonstrated 9 different genotypes: *SENV-A* to *SENV-H* (Kojima et al., 2003). *SENV-D* and *SENV-H* genotypes are related to transfusion-associated non-A to E hepatitis (Mikuni et al., 2002) and are more prevalent within the population exposed to transfusion (Kao et al., 2002).

SEN-V-B, -A and -E have been less frequently found among blood donors and do not appear to be related to non A to E hepatitis. On the other hand, genotypes D and H have been detected in 1% of blood donors but in more than 50% of non A to E hepatitis cases (Serin et al., 2005). The co-infection with HCV is considered a risk factor for *SEN-V* infection and the exact interaction of this virus with hepatitis C virus (HCV) and hepatitis B virus (HBV) is still unclear (Tahan et al., 2003). Furthermore, the prevalence of *SEN-V* increases in association with *HIV-1* and hepatitis B and C viruses. This strongly supports the hypothesis that *SEN-V* is transmitted via blood (Umemura et al., 2001). 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 (Mu et al., 2004).

The purpose of present study was to determine the prevalence of *SEN* virus infection in blood donors and patients with chronic HBV and HCV for the first time in Chaharmahal Va Bakhtiari province located in southwest Iran.

MATERIALS AND METHODS

Serum samples and study populations

The serum samples were obtained from 240 patients with chronic HBV and HCV (172 HBV and 68 HCV) and 60 non-professional blood donors from the blood transfusion organization, hospital and clinical and pathological laboratories in Chaharmahal Va Bakhtiari province located in southwest Iran. All serum samples were stored frozen at -20°C until analysis.

Viral DNA extraction

The viral nucleic acid was extracted from 200 µl serums using a QIAamp DNA Blood Mini Kit (Qiagen Ltd, Crawley, UK) according to the manufacturer's recommendation. The extracted genomic DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm according to the method described by Sambrook and Russell (2001).

Detection of *SENV-D* and *SENV-H* genotypes by PCR

The presence of *SENV-H* and *SENV-D* DNA was determined by polymerase chain reaction (PCR) with type-specific primers according to the research articles by Umemura et al. (2001) and Kojima et al. (2003). These primers sequences (D10S: 5´-GTA ACT TTG CGG TCA ACT GCC-3´, L2AS: 5´-CCT CGG TT[G/T] [C/G]AA A[G/T]G T[C/T]T GAT AGT-3´, C5S: 5´-GGT GCC CCT [A/T]GT [C/T]AG TTG GCG GTT-3´ and L2AS: 5´-CCT CGG TT[G/T] [C/G]AA A[G/T]G T[C/T]T GAT AGT-3´) were used for *ORF1* gene amplification of *SENV-D* and *SENV-H*, respectively.

PCR was carried out in 25 µl total reaction volumes, each containing 100 ng of template DNA, 0.2 pM of each primer, 2.5 µl of 10X PCR buffer, 1.5 mM $MgCl_2$, 200 mM dNTPs, and 1 unit of *Taq* DNA polymerase (Fermentas, Germany). The amplification reaction consisted of 5 min of pre-denaturing at 94°C, followed by 32 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, and 1 min extension 72°C, and then by a final extension at 70°C for 5 min. The samples were amplified in a Gradient Palm Cycler (Corbett Research, Australia). The PCR amplification products (10 µl) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with Ethidium Bromide, and images were obtained in UVIdoc gel documentation systems (UK).

Statistical analysis

Analysis of data was performed using the SPSS version 17.0 computer software (SPSS, Chicago, IL). Also, the differences between prevalence of *SEN-V* infection in chronic hepatitis patients and healthy blood donors were examined by T test statistical analysis. P values <0.05 were considered significant.

RESULTS

Analysis of PCR products for the presence of *SENV-H* and *SENV-D* DNA on 1% agarose gel revealed 229 base pairs (bp) for *SENV-H* and 222 bp for *SENV-D* (Figure 1). *SENV-H* and *SENV-D* DNA was found to be positive in 54/172 (31.39%), and 48/172 (27.91%), of hepatitis B patients and 23/68 (33.82%), and 27/68 (39.7%) of patients with hepatitis C, respectively. In the control group, *SENV-H* and *SENV-D* was detected in 8/60 (13.33%) and 6/60 (10%) of the healthy blood donors, respectively (Table 1).

DISCUSSION

SEN-V was first reported on July 20, 1999, in the serum of a human immunodeficiency virus type 1 (*HIV*-1)-infected patient possessing hepatitis with unknown etiology in Italy, and was named on the basis of initials of the patient (Yoshida et al., 2002). The prevalence of 5 out of 9 *SEN-V* strains (A, B, C, D, H and E) as well as a consensus sequence as total *SEN-V* have been studied in various donor and patient populations. Among nine genotypes of *SEN-V* (A to I), 5 strains (A, B, H, C, D and E), as well as a consensus sequence and *SENV-H* and *SENV-D* genotypes were extremely associated with non-A to E hepatitis (Schroter et al., 2003). It has been suggested that genotypes *SEN-V* C and H as well as *SEN-V* D and F could be combined due to similarities in ORF 1 (Umemura et al., 2001). Tanaka et al. (2001) found

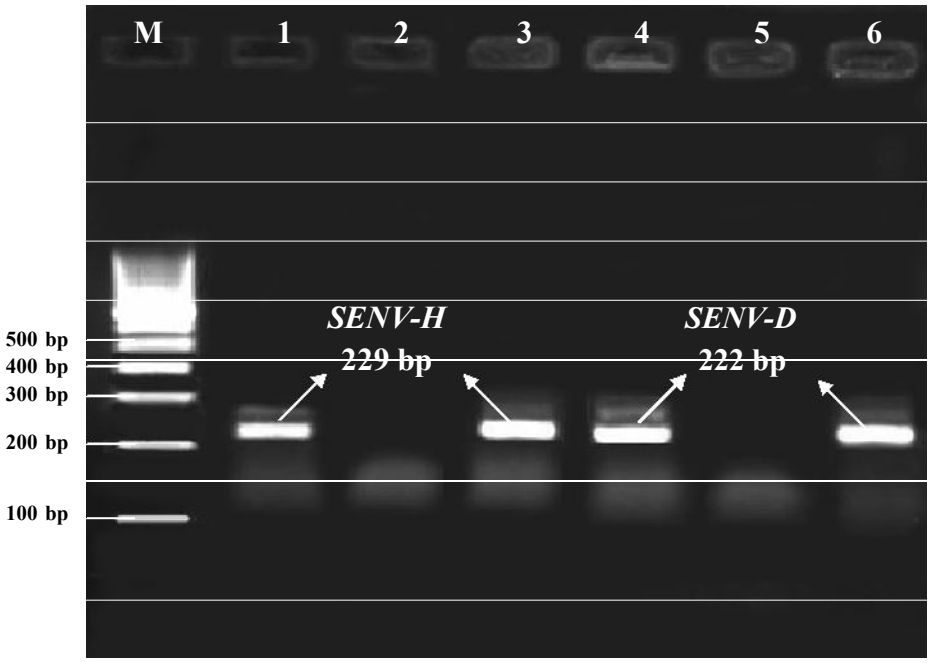

Figure 1. Gel electrophoresis for identification of *SENV-H* and *SENV-D*. Line M: 100 bp molecular weight markers (Fermentas, Germany), lines 1 and 3: 229 bp PCR products of *SENV-H* DNA, line 2: *SENV-H* DNA negative sera sample, lines 4 and 6: 222 bp PCR products of *SENV-D* DNA, and line 5: *SENV-D* DNA negative sera sample.

Table 1. Prevalence of *SEN-V* infection in patients with chronic *HBV* and *HCV* in compared with control groups (healthy blood donors) in southwest Iran (Chaharmahal Va Bakhtiari province).

Patients	Samples	Both *SENV-H*/D positive no. (%)	*SENV-H* positive no. (%)	*SENV-D* positive no. (%)
HBV	172	102 (59.3)	54 (31.39)	48 (27.91)
HCV	68	50 (73.52)	23 (33.82)	27 (39.7)
Controls (healthy blood donors)	60	14 (23.33)	8 (13.33)	6 (10)
Total	300	166 (55.33)	85 (28.33)	81 (27)

that *SEN-V* and *TTV* had similar structure. *SEN-V* has homogeneity of 55% at nucleotide level with *TTV*, but they only have homogeneity of 37% at amino acid level.

The prevalence of *SENV-H* and D in present study was 31.39 and 27.91% in chronic hepatitis B patients and 33.82 and 39.7% in chronic hepatitis C patients, respectively. Furthermore, *SENV-H* and D was detected in 13.33 and 10% of healthy blood donors. Results showed high prevalence of *SEN-V* genotype H and D in patients with chronic HBV and HCV compared blood donors in southwest Iran (Chaharmahal Va Bakhtiari province). The significant differences for prevalence of *SEN-V* infection between the hepatitis patients and healthy blood donors group using T test statistical analysis ($P<0.05$) were observed in this study. The high prevalence of *SEN-V* infection in hepatitis patients in comparison to blood donors possibly is due to the same risk factors for these viruses such as blood transfusion in this region.

The distribution of *SENV* strains D and H among hepatitis patients, blood donors and other healthy populations varied in different regions. Also, *SEN-V* was found not to be practical because the prevalence in donors was 13% and the rate in a transfused population exceeded 70% (Umemura et al., 2001).

Umemura et al. (2001) showed the strong association of *SEN-V* with transfusion-associated non–A to E hepatitis and it is similar to the results of the present study. The prevalence of *SEN-V* in healthy blood donors from various geographic areas such as Japan (10 to 22%) (Shibata et al., 2001), Taiwan (15%) (Kao et al., 2002), Thailand (5%) (Tangkijvanich et al., 2003), United States (1.8%) (Umemura et al., 2001), Germany (8 to 17%) (Schroter et al., 2002), and at least 13% in Italy (Pirovano et al., 2002). These findings were same to the results of the present study which confirmed it. Mikuni et

al. (2002) found *SEN-V* infection with a high prevalence among blood donors and none had a history of serious illness or blood transfusion. In their study, the less considerable frequency of blood transfusion in subjects with non-B, non-C hepatitis liver disease than in those with chronic HCV-related liver disease, which suggests the existence of non-transfusion related routes. The study of Mu et al. (2000) in China showed that 31% of the blood donors had *SENV-D*/*H* DNA and it was higher than those in America and Italy (2%), and in Japan and Taiwan (15 to 20%). The prevalence of *SENV-H* among Taiwanese chronic hepatitis C patients with combination therapy of high-dose interferon-alfa and ribavirin was 19.2% (Dai et al., 2004). In the study of Omar et al. (2008) on Egyptian patients with hepatitis C virus related chronic liver disease and patients undergoing hemodialysis, *SEN* virus-D/H DNA was detected in 13.5% of patients with chronic liver disease, 11.1% of patients undergoing hemodialysis, and 7.1% of healthy controls. They showed no significant differences between patients and the control group. The study of Sharifi et al. (2008) on prevalence of *SEN-V* infection in Iranian blood donors showed that 4 (1.5%) of the 260 were infected. *SENV-H* viremia was detected in 47 (18.08 %) of the 260 blood donors and both *SENV-D* and *SENV-H* viremia were detected in 9 (3.4%) of the 260 blood donors. Furthermore, in total *SENV-D* or *SENV-H* viremia was identified in 60 (23.08%) of the 260 blood donors. This results also showed that the high prevalence of *SEN-V* in healthy blood donors with no history of blood transfusion in Iran (Sharifi et al., 2008). The results of their study confirmed the high prevalence of this virus that observed in current research. The study of Karimi-Rastehkenari and Bouzari (2010) in Iran showed that frequency of *SEN-V* strains (*SENV-H* or *SENV-D*) and co-infection (both *SENV-D* and *SENV-H*) viremia was significantly higher among thalassemic patients than healthy individuals.

The results of the current study indicate that PCR amplified with genotype-specific primers could be useful for detection and screening *SENV-H* and D genotype. Furthermore, the findings of the present study in southwest Iran demonstrate that *SENV-D* and *SENV-H* was detected in a high proportion of patients with chronic HBV and HCV compared with healthy blood donors.

In conclusion, the results of present study showed that *SENV-H* and D are more prevalent in hepatitis patients in comparison with blood donors in our region. This virus is parenterally transmitted, and therefore, its spread could be controlled by appropriate screening of hepatitis patients, blood donors and blood products.

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