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Original article

The prevalence of SEN virus among blood donors in the Eastern Province of KSA

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1. Introduction

Many previous studies have suggested that other hepatitis agents/viruses besides ordinary A to E hepatitis viruses have been existing (Alter et al., 1989; Alter and Bradley, 1995). After detecting many patients with post-transfusion hepatitis with unknown origin confirmed negative for the regular viruses of hepatitis (Alter and Bradley, 1995; Alter et al., 1992). The unknown agent/virus has also been implicated in a significant percentage of acute liver failure, chronic hepatitis, and liver cirrhosis (Umemura et al., 2003). Therefore, several investigations carried out to detect that unknown causative agent/virus. These investigations were firstly suggested hepatitis G virus (HGV) (Linnen et al., 1996), TT virus (Nishizawa et al., 1997), and SEN virus (SEN-V) as main agents of the hepatitis of unknown cause (Matsumoto et al., 1999; Tanaka et al., 2000).

SEN virus (SEN-V) is one of the widely distributed blood-borne virus (Quasim and Alshaikhly, 2019). It was discovered in 1999 in Italy as a new virus isolated from the serum of intravenous drug users (Tanaka et al., 2000; Yoshida et al., 2002). It proposed that SEN-V involved in post-transfusion hepatitis as it found in the sera of patients with unknown origin hepatitis (non-A-E hepatitis) and quietly has a low frequency in the sera of normal blood donors (Karimi-Rastehkenari and Bouzari, 2010; Pirouzi et al., 2014). Two of them (SEN-V-D and SEN-V-H) have been described as potential elect viruses for motivating post-transfusion hepatitis. Until now, it is unclear whether patients on maintenance hemodialysis are at increased risk for acquiring the SEN virus. SEN-V-D and SEN-V-H were also detected in patients with fulminant hepatitis, which confirms that those two types may participate in causing liver diseases (Thom et al., 2011). The thalassemic patients who have a high level of SEN-V prove the route of blood transfusion (Karimi-Rastehkenari and Bouzari, 2010). It is a non-enveloped virus (Amer et al., 2020; Pirouzi et al., 2014) belonging to the family Circoviridae (Dehkordi and Doosti, 2011). The SEN-V genome consists of approximately 3.8 Kb of a circular single-stranded DNA molecule (Amer et al., 2020; Pirouzi et al., 2014). The SEN-V genome show similarity of 55% in nucleotide sequences with Torque Teno virus, which belongs to a new family called Anelloviridae (Davidson et al., 1999; Nishizawa et al., 1997; Sagir et al., 2004), make the SEN-V have a strong relationship with this new virus family (Khudair et al., 2019). There are nine different genotypes of the SEN-V (A through I) (Karimi-Rastehkenari and Bouzari, 2010), which differ by at least 25% in the nucleotide sequences (Kojima et al., 2003).

The majority of research papers have reported on D and H genotypes as clinically significant strains. They are the only factors detected in patients with unknown hepatitis (non-A to E hepatitis) and healthy blood donors but less commonly (Abbasi et al., 2016; Karimi-Rastehkenari and Bouzari, 2010; Pirouzi et al., 2014).

SEN-V prevalence varied among the different geographic region. Its prevalence among healthy control individuals in the USA was 2–3% compared with the high-frequency level (30%) among recipient of blood transfusion (Umemura et al., 2001), which prove the transmission of the virus through blood (Karimi-Rastehkenari and Bouzari, 2010; Umemura et al., 2001), as demonstrated by comparing the sequence homology between donors and recipients (Shibata et al., 2001).

Asymptomatic carriers of the SEN-V may persistently infect other individuals in a period exceeding 12 years (Quasim and Alshaikhly, 2019). This study aimed to determine the prevalence of SEN-V and its genotypes D and H among healthy blood donors in the Eastern Province of KSA.

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2. Materials and methods

2.1. Ethical approvalaaa

This study was reviewed and approved by the Institutional Review Board (IRB), Prince Sultan Military College of Health Sciences, Dhahran, KSA (IRB-2017-CLS-002). A written informed consent obtained from each blood donor.

2.2. Study design and subjects

This study had a cross-sectional design and included 315 healthy blood donors (31 females and 284 males) from the eastern province of KSA, extending from October 2018 to January 2020. The sera of all donors were negative for HBsAg, anti-HCV antibodies, and anti-HIV antibodies.

2.3. Blood samples collection

Ten millilitres of whole blood was collected into sterile gel tubes without anticoagulant (red top) from each participant and allowed to clot. Then the serum was separated by centrifugation at 3000 rpm, divided into aliquots, and stored at −80 °C until testing.

2.4. DNA extraction from sera

According to the manufacturer's instructions, DNA extraction was carried out from 200 μl serum using DNA Isolation mini Kit (Norgen Biotek Corp, Ontario, Canada. Cat. # 46380). The extracted DNA stored at −80 °C before testing.

2.5. SEN virus DNA amplification and detection by nested PCR

The SEN-V amplification was done by nested polymerase chain reaction (PCR) by using two rounds of PCR runs according to (Hosseini and Bouzari, 2016; Kojima et al., 2003) with protocol modification for the optimization of PCR results. The first round of nested PCR used pair of primers, forward primer AI-1F (5′-TWCYCMAACGACCAGCTAGACCT-3′) and reverse primer AI-1R (5′-GTTTGTGGTGAGCAGAA CGGA-3′), which amplified a conserved region for all SENV genotypes (A-I) that consist of 349-bp (Hosseini and Bouzari, 2016). A 25 μl reaction mixtures were prepared by mixing 12.5 μl of an Absolute Master Mix (MOLECULE), with 1 μl of each of the forward and reverse primers of 10 μM/μl (SEN-V AI-1F and SEN-V AI-1R), 5 μl of the extracted DNA template, and finally 5.5 μl of Nuclease-free sterile water. All the samples, including the negative and positive controls, were subjected to a thermocycler (Bio-Rad, USA) and programmed with the following conditions: one cycle at 94 °C for 5 min; 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min for 35 cycles, with a final extension at 72 °C for 10 min for 1 cycle.

For the second round of the nested PCR, specific primers, forward and reverse primers for the of 195-bp SEN-V D which included D-1148F (5′- CTAAGCAGCCCTAACACTCATCCAG-3′) and D1341R (5′-GCAGTTGACCGCAAAGTTACAAGAG-3′). The forward and reverse primers for SEN-V H included H-1020F (5′-TTTGGCTGCACCTTCTGGTT-3′), and H-1138R (5′-AGAAATGATGGGTGAGTGTTAGGG-3′) were used to amplified a sequence length of 119-bp of the H phenotype. A similar PCR reaction mixture of 25 μl was prepared with the difference that 5 μl from the product of the first round was used as a template, and the following PCR conditions were programmed: one cycle at 94 °C for 5 min; 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min for 30 cycles, with a final extension at 72 °C for 10 min for 1 cycle.

The second runs' final product was detected using 2% agarose gel electrophoresis after the addition of ethidium bromide stain. The presence of any products with 195-bp and 119-bp indicated SEN-V D and H genotypes, respectively (Abbasi et al., 2016).

3. Results

The demographic information of 315 blood donors was summarized in Table 1. The study group were healthy blood donors, ranging in age from 18 to 55 years. The prevalence of SEN-V among the 315 blood donors using nested PCR following agarose gel electrophoresis was 10.9%. In contrast, the prevalence of SEN-V genotypes D and H were 0.6% and 10.2%, respectively. The prevalence of SEN virus H genotype was 17-fold higher than that of SEN virus D genotype in healthy blood donors (Table 2). There were no co-infection cases of SENV-H + SENV-D among the blood donors. The product of the nested PCR on agarose gel electrophoresis indicated a 195-base pair band for SENV-D and a 119-base pair band for SENV-H (Fig. 1).

4. Discussion

According to the reviewed literature, the SEN-V is highly related to the new Anellovididae virus family (Khudair et al., 2019; Sagir et al., 2004) because they found a 55% nucleotide sequences homology between the SEN-V genome and TT virus, which belong to the new family Anelloviridae (Davidson et al., 1999; Sagir et al., 2004). For the first time, the Anelloviridae family was recognized as the cause of non A-E viral hepatitis in 1997 (Nishizawa et al., 1997). However, their pathogenicity in causing hepatitis has not been settled and need to be studied more.

Many studies have revealed a variation in the prevalence rate of SEN virus among apparently healthy people of various populations, which may be due to the difference between these populations and the recent advancement of the detection methods and techniques (e.g. molecular methods).

Only two out of the nine genotypes of the SEN-V, namely SENV-D and SENV-H, are considerably associated with transfusion-related non-A to E viral hepatitis (Umemura et al., 2001). Moreover, the significant statistical association between SENV (H and D genotypes) and post-transfusion hepatitis increased the probability that SEN virus is one of the possible infectious agents of hepatitis of unknown origin beside hepatitis G virus and TT virus. Different previous studies proved that both genotypes have a high incidence rate in patients with a transfusion history than those healthy blood donors (Mushahwar, 2000; Umemura et al., 2001). This information about these viruses allows us to establish the SEN virus prevalence in healthy blood donors for the first time in Saudi Arabia.

The present report's findings revealed that the SEN-V DNA was identified in the sera of 34 individuals out of 315 healthy blood donors (10.8%) in the Eastern Province of KSA. These results were consistent with the prevalence of the virus isolated from healthy blood donors in Iraq (10.7%) (Khazaal et al., 2016), in Japan (10%) (Shibata et al., 2001), in Italy (13%) (Pirovano et al., 2002a,

Table 1
The demographic information of 315 blood donors.

Variable	Frequency
Age (years)	Range 18–55
Gender	Male = 284 (90.2%) Female = 31 (9.8%)

Table 2
The prevalence of SEN-V and it infection in 315 blood donors in the eastern province of KSA using nested-PCR.

Variable	Positive	Negative
SEN-V DNA	34/315 (10.8%)	281/315 (89.2%)
SENV-H genotype	32/315 (10.2%)	283/315 (89.8%)
SENV-D genotype	2/315 (0.6%)	313/315 (99.4%)
Coinfection (SENV-D & H)	0/315 (0%)	No coinfection

2002b), in Taiwan (15%) (Kao et al., 2002), and in Germany (8–17%) (Schröter et al., 2002).

In the present study, the prevalence of the SEN virus infection among healthy blood donors was significantly lower than its prevalence in healthy adults reported in Iran by Karimi and Bouzari (90.8%) (Karimi-Rastehkenari and Bouzari, 2010) and by Hosseini and Bouzari (90.5%) (Hosseini and Bouzari, 2016); in Japan (75%) (Gerner et al., 2000); in China (31%) (Mu et al., 2004); in Southern Taiwan (24.2%) (Dai et al., 2004); and in Greece (24%) (Umemura et al., 2003). The reasons for these differences in SEN virus prevalence rate between populations are not clear. However, the differences can be described in some ways. One of the main reasons is the difference in the demographic characteristics of each population. The gender distribution of this study showed an increased number of males than females (284 Males and 31 Females) as normally observed in blood banks and transfusion centres of Saudi Arabia.

In our study, the overall frequency of this virus among blood donors was slightly higher than the prevalence rate reported in the USA (1.8%) (Umemura et al., 2001), and in Thailand (5%) (Tangkijvanich et al., 2003). This virus's geographic distribution rate among healthy individuals shows wide variation ranging from only 1.8% in USA (Umemura et al., 2001) to 90.8% that reported in Iran by Karimi-Rastehkenari and Bouzari (2010). The probable reason for these differences is the variations of the population's characteristics.

Regarding the percentage of SENV-D and H variants among healthy individuals, the prevalence of this study's SENV-H genotype was 10.2%, which was 17-fold higher than that of the SENV-D genotype (0.6%). Our result about the predominant of SENV-H higher the SENV-D genotype appears to agree with many studies done in different countries around the world in Turkey (Serin et al., 2006, 2005), in Iran (Abbasi et al., 2016; Pirouzi et al., 2014), in Iraq (Khudair et al., 2019), in Scotland (Thom et al., 2011), in Germany (Umemura et al., 2003), in Ghana (Thom et al., 2011), in Thailand (Tangkijvanich, 2003), and Czech Republic (Thom et al., 2011). However, These results were inconsistent with few other studies reported in Egypt (Mohamed et al., 2011), in Japan (Umemura et al., 2003), in China (Mu et al., 2004), in Taiwan (Dai et al., 2005, 2004), and in Greece (Umemura et al., 2003). Their findings show SENV-D to be more abundant than the SENV-H variant.

5. Conclusions

Our findings of the overall SEN-V prevalence was consistent with the majority of previously published researches. However, minor differences in the prevalence of D and H genotypes have been identified, which might be due to the variations between different regions population and other routes than parenteral transmission. These points may need further investigations.

In conclusions, for the first time in KSA, we have recognized the prevalence of the SEN-V and its clinically important genotypes D and H among healthy blood donors. Their prevalence in Eastern KSA were 10.8%, 0.6%, and 10.2%, respectively. The SENV-H genotype was more prevalent than the SENV-D by 17-folds in the study participants.

Generally, the SEN virus's overall prevalence rate in the Kingdom of Saudi Arabia is considerably low. However, the hazard of blood transmission of the SEN virus remains important. So, it is crucial to conduct future research projects to determine the

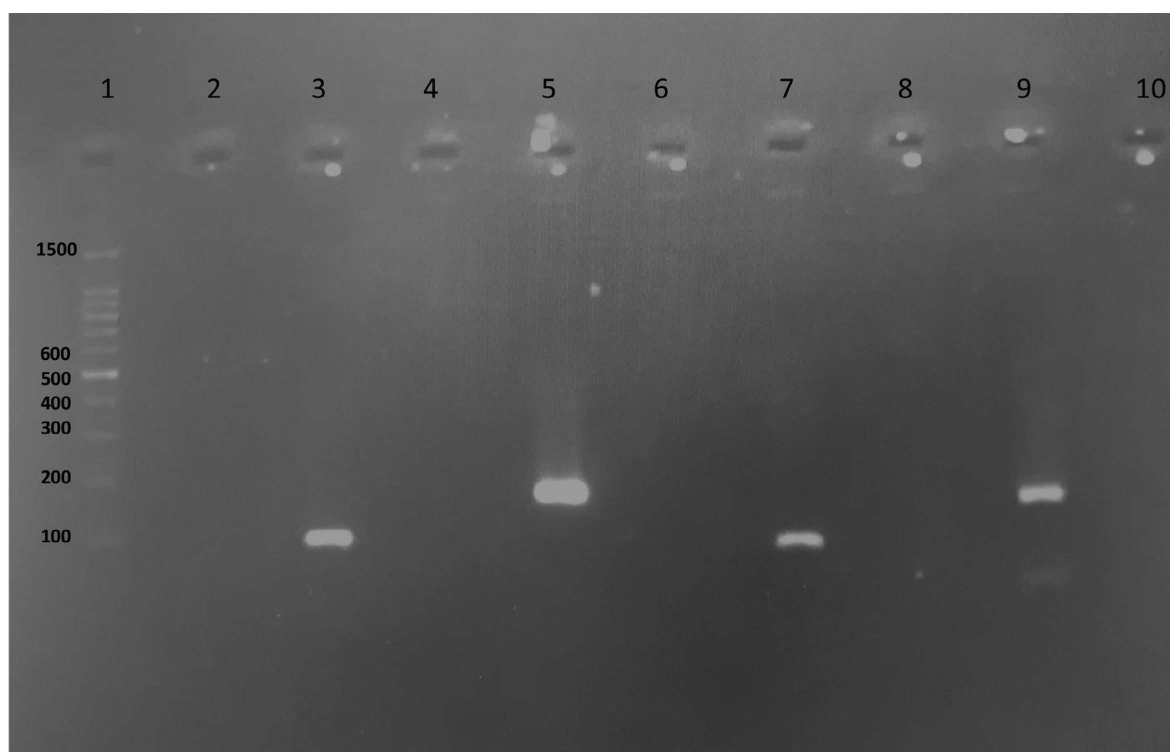

Fig. 1. Electrophoresis of the nested PCR Product. Lane 1, ladder (100 base pairs); Lane 2, negative control; Lane 3, SENV-H positive control; Lane 4, 6, 8, and 10 negative samples for both SENV-D and H; Lane 5, SENV-D positive control; Lane 7, SENV-H positive sample; and Lane 9, SENV-D positive sample.

pathogenicity and clinical significance of SEN virus to assess the usefulness of screening Saudi blood donors for SEN virus to reduce the transmission risk of these agents in blood donations to a minimum to get "zero risk" blood.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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