

THE PREVALENCE OF SEN VIRUS GENOTYPES IN BAGHDAD CITY

Tariq M. Quasim, Abdelwahab A R. Alshaikhly

Department of pharmacy, Al-Rasheed University College, Baghdad, Iraq.
E. mail: Tarikmkasim1956@gmail.com, Wahabshaikhly@yahoo.com

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ABSTRACT

SEN virus (SEN V) is one of worldwide incidence of blood borne pathogen. The most dominant genotypes among the nine genotypes of the SEN virus are SENV-D and H. The objective of this research was to determine the frequency of SENV genotypes particularly SENV- D and H genotypes in healthy Iraqi participant's sera. Totally, 200 serum samples from healthy voluntaries were collected from both gender as well as subjected to nested PCR. SENV-D was detected in 30% healthy individuals and more frequent in tested male 51.25 than in female individuals 48.75%. The frequency of SENV-H was found in 5% of participants. SENV-D is the dominant strain in the Iraqi studied group followed by genotypes H. phylogenetic tree analysis of virus near-complete open-reading frame 1(ORF1), was performed, and the evolutionary relationship indicated that local isolates were very close to the group that included the Asian isolates.

Key words: SEN virus, genotypes H, genotypes D, PCR, Sequence,

INTRODUCTION

SEN virus is a non-enveloped circular single-stranded DNA (ssDNA) virus considered as one of the blood-borne infections agents (Pirouzi et al., 2014). It is a member of superfamily of TTV-related viruses (Tanaka et al., 2001). Its genome organized in three open reading frames (ORF) sized 3600–3900 nt (Mushahwar, 2000; Bowden, 2001; Tanaka et al., 2001). Nine genotypes (A-I) have been described (Karimi-Rastehkenari, and Bouzari, 2010) with high sequence diversities ranging between 15-50% among these genotypes (Kojima et al., 2003). Genotypes H and D in both blood donors and in hepatitis patients are the most associated genotypes with the pathogenesis virus of non-A - E hepatitis and post-transfusion hepatitis (Abbasi et al., 2016). The SEN V infection effect on chronic viral hepatitis situation is not very clear till now (Shalan et al., 2010; Elsherbiny et al., 2015; Hosseini and Bouzari, 2016). Molecular detection of pathogens still best way (Al-Ammar, and Al Maghathry, 2017). Both H and D Genotypes are dominant strains in different regions of the world. In the United States the prevalence of D or H genotypes among blood transfusion recipients was 30%, in compared with 2-3% among control participants (Umemura et al., 2001). As result in a persistent state of SEN V it is supposed to be highly prone to mutation (Dehkordi and Doosti, 2011). The SEN V caused persistent infection that may exceed from 1-12 year (Umemura et al., 2002). Reinfection with SEN V different strains may occur in retested specimens after a median of 9.3 years. The virus was still present in 61.0% of subjected patients (Wilson et al., 2001).

Our study was focusing on the prevalence of SEN V and to determine the predominant genotypes in Iraqi patients.

MATERIALS AND METHODS

Viral DNA isolation: Two hundred samples of human serum will be collected from healthy participants from Baghdad province during a 3 months period from July to October 2018. Viral DNA was extracted from 140µl serum samples using QIA-amp® virus kits (Qiagen, Germany) according to the manufacturing instruction.

Detection of SEN virus DNA: SEN virus DNA was detected by nested conventional PCR using specific three sets of primers synthesized and supplied by IDT (Integrated DNA Technologies company, Canada) according to Kojima et al., (2003). The first run of PCR was conducted with a primer A11F and A11R pair that amplified 349-bp of conserved region for all 9 SEN V genotypes from (A-I). PCR reaction mixture volume was 25 µl containing 2µl of viral genomic DNA, 12.5µl GoTaq0T® Green Master (Promega, USA), 1µl of 10Pmol/µl of primer mix work solution, 0.5µl of 25mM MgCl₂, 9µl of nuclease free distilled water. Amplification program was 1 cycle at 94°C for 5 min; 35 cycles of 94°C for 30s, 55°C for 30s, 72°C for 1min; 1 cycle at 72°C for 10min, using the Master cycler PCR (Eppendorf, Germany). Nested PCR second round was conducted using 2µl of PCR product from first around D1148F, D1341R for SEN V-D genotype, H-1020F H-1138R for SEN V-H genotype with same master mixture condition that mentioned above. The amplification program was involved a pre denaturation 1 cycle at 94°C for 2 min and 30 cycles

(30s at 94°C, 30s at 56°C and 1 min at 72°C, then final extension for 1 cycle for 10 min at 72 °C) for both SEN V-D and SEN V-H with a 10-min final extension at 72°C. The PCR product was subjected to 2% agarose gel electrophoresis, and visualized under UV537nm (Imagemaster VDS, Pharmacia Biotech, USA) after stained with Red Safe Nucleic Acid (RSNA) staining solution (20,000x). The PCR products 25µl from the second round in addition to 10µl of 10Pmol/µl of each primer were sent to Macro-gene Company (Korea) by AB DNA Sequencing system. Phylogenetic tree analysis of near-complete open-reading frame 1 (ORF1) was performed. Sequence data related to the gene on representative strains of the various genotypes were retrieved from NCBI GenBank and included in the sequence alignment and phylogenetic analysis had processed in MEGA 6. The analysis involved about 20nt sequences (Tamura et al., 2013). The final data sets were including of 138 nucleotide positions. The evolutionary history was deduced by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method (Sneath and Sokal, 1973).

Table1: Primers were used to amplified GB partial gene by nPCR

Primers	Oligonucleotide sequence	Amplicons Size
First a round nested PCR primers		
A11F	5-TWCYCMAACGACCAGCTAGACCT-3	349 nt
A11R	5-GTTTGTGGTGAGCAGAACGGA-3	
D1148F	5-CTAAGCAGCCCTAACACTCATCCAG-3	198 nt
D1341R	5-GCAGTTGACCGCAAAGTTACAAGAG-3	
H1020F	5-TTTGGCTGCACCTTCTGGTT-3	124 nt
H138R	5-AGAAATGATGGGTGAGTGTAGGG-3	

RESULTS

The 200 blood donor's samples were taken from male and female with ages ranged between 20 - 58 years old, the mean age is 38 years old. The frequency of the viral infection in healthy individuals is shown in Figure 3. The nPCR results was obtain after agarose gel electrophoresis run which indicated a band sized 198-bp for SENV-D and a band sized 119bp for SENV-H (Figure 1,2). About 30% (60 out of 200) of the healthy individuals were infected with the SENV-D as well as in 31/60 (51.25%) in male individuals and 29/60 (48.75%) in female individuals, on the other hand the SENV-H was detected in 10 participant only which represented 5% (10 out of 200), about 5/10 (50%) of both male and female. The prevalence of genotype SENV-D appears 6-fold higher than that recorded in SENV-H in participants. The branch length of tree is equal to 0.41097765 is shown in figure 1. The evolutionary distances were computed using the Maximum Composite Likelihood method as mentioned by Tamura et al (2004).

Moreover, the blasted SENV Iraqi isolates sequences were 96%-99% identical to SENV sequences in NCBI. The phylogenetic tree including two main clads the upper one represented group A which contains the Iraqi detected strains sequences marked with blue triangle figure 4. All Iraqi strains were found identical 100% to the Iranian SENV gene bank accession AB856065.1, and 99 % identical with other Asian strains that isolated from Iran, China, and Japan. On the other hands it seems less identical 96% with USA, and Brazil isolates in North and South America with branch distance = 0.1137.

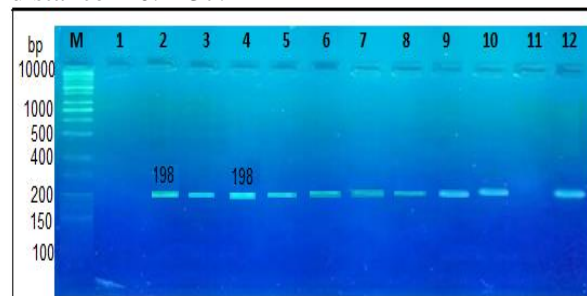


Figure 1: Gel electrophoresis profile of nested PCR products of capsid gene of SEN virus Using 2 % agarose stained with red safe DNA dye and electrophoresed by 5vol/cm in TBA buffer. Line M represent ladder DNA 100-10000 bp, line 1 negative control, Lane 2-12 the amplicons sized 198 bp.

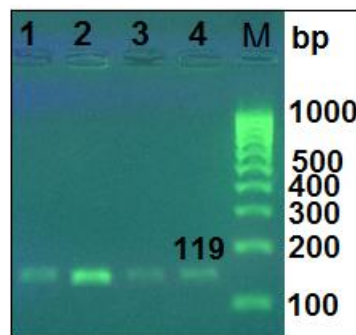


Figure 2: Gel electrophoresis profile nested PCR products of capsid gene of SEN virus using 2 % agarose stained with red safe DNA dye and electrophoresed by 5vol/cm in TBA buffer. Line M represent ladder DNA 100-1000 bp, line 1 -4 the amplicons sized 119 bp.

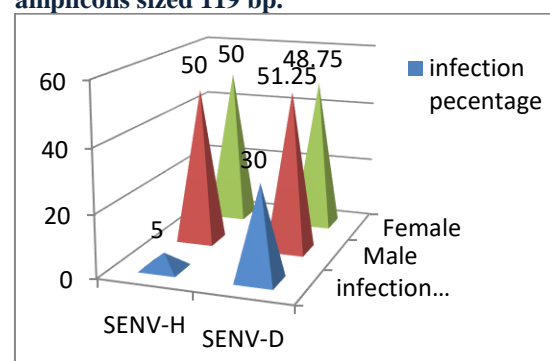


Figure 3: SEN virus infection among healthy participants in both females and males

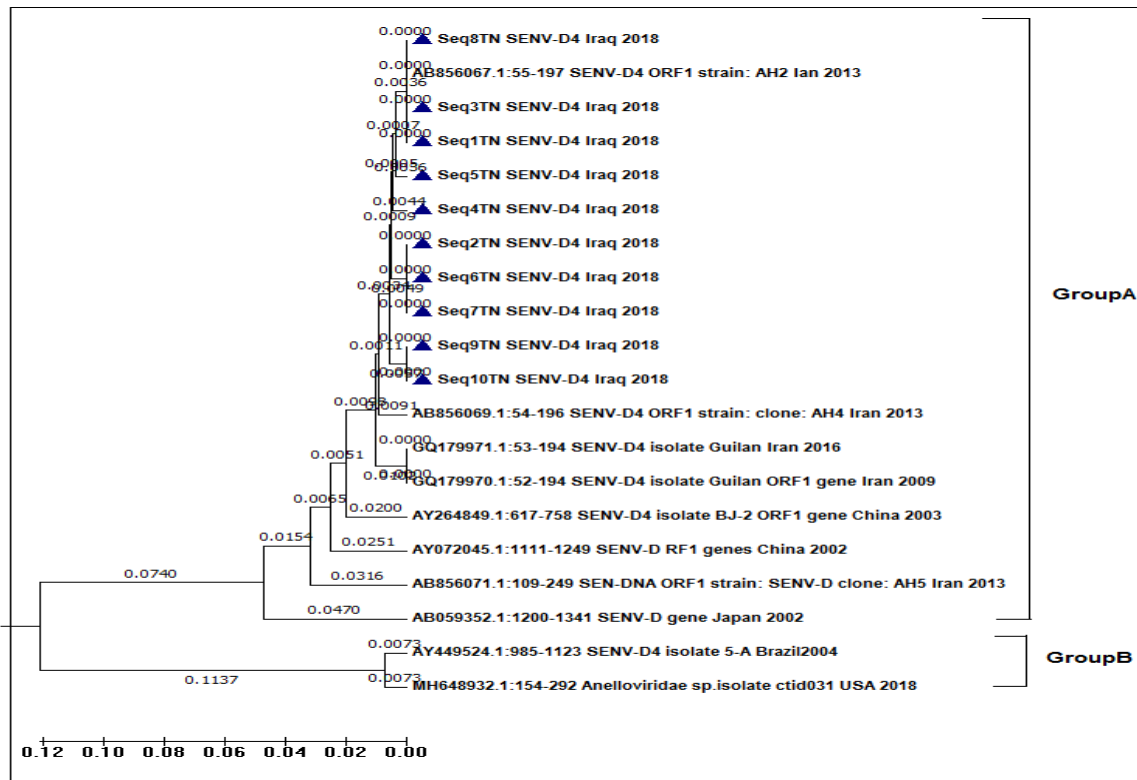


Figure 4: The phylogenetic tree of SEN virus partial ORF1 gene inferred using the UPGMA method. Iraqi isolates marked with blue triangles rooted in compared with NCBI sequenced data. The Iranian SEN virus strain accession AB856065 represented the rooted sequence. Evolutionary analyses were conducted in MEGA6 software.

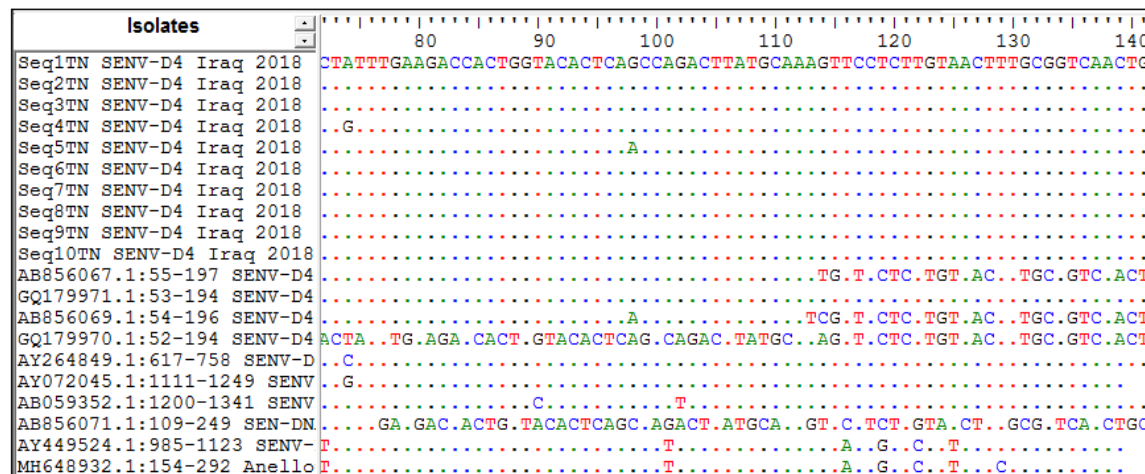
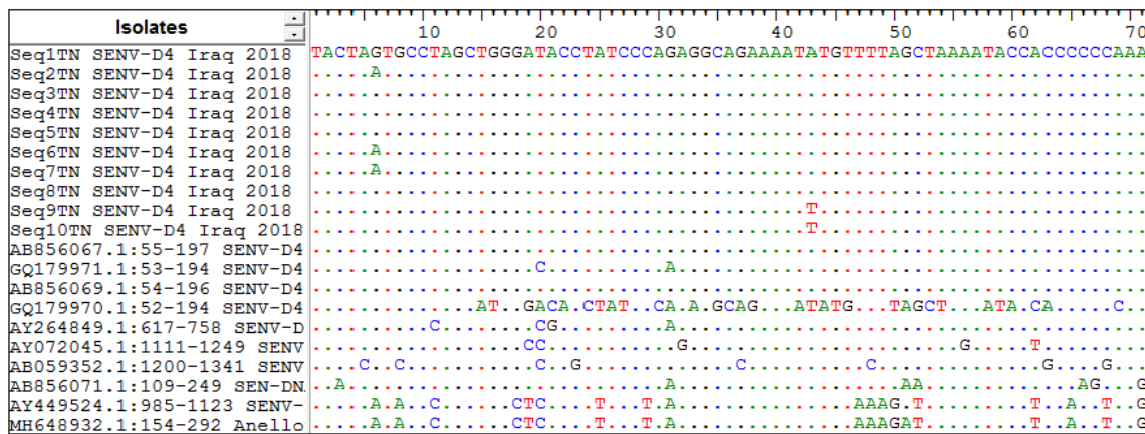


Figure 5: The nucleotide variation in SEN virus partial ORF1 gene

DISCUSSION

The rates of prevalence of this virus vary from country to country according to published studies. Our findings are consistent with the incidence of the virus (31%) has been reported previously in China (Mu et al., 2004) with the findings of 24% SENV-D and SENV-H reported in Greece (Umemura et al., 2003) and also the results of 23.08% that were reported by Sharifi et al., (2008) in Tehran, Germany (17%) (Schroter et al., 2003), Taiwan (15%) (Kao et al., 2002), Italy (13%) (Pirovano et al., 2002), and Thailand (5%) (Tangkijvanich et al., 2003). The prevalence of SEN V strains D and H among healthy blood donors varied from a low of 1.5% in the United States (Umemura et al., 2001) to a high of 90.8% in Iran (Karimi and Bouzari 2010). Our finding is more consistent with that reported by Karimi-Rastehkenari and Bouzari (2010) confirms the higher frequency of the virus in healthy individuals in Guilan Province in Iran compared to other countries. Also Hosseini and Bouzari (2016) refers to that the SEN V in healthy individual was more frequent 90% than in HBV 66%, and HCV 46% positive individuals, The frequency of genotype SENV-H in healthy individuals was higher than SENV-D, and our results are in agreement with many studies over world (Serin et al., 2005, Serin et al., 2006) finding in Turkey, Kao et al., (2002) in Taiwan reported SENV-H to be more prevalent than SENV-D (Tezcan et al., 2009) but not consistent with that data reported by Mohamed et al., (2011) in Egypt about the predominant of SENV-D genotype in all SENV positives of control group, and from Japan, which detected SENV-D in 77% and SENV-H in 15% of the participants (Kobayashi et al., 2003). Because of the lack of studies on the prevalence of the SENV infection and genotyping in Iraq and the limited submission sequences of this virus on the National Center of biotechnology Information (NCBI), the comparison with standard isolates is represented a challenge to us (Abbasi et al., 2016) found that 24% of tested blood donors in Ahvaz City were positive for both SENV-D and SENV-H. (Yoshida et al., 2002) in their study on Japanese patients and (Kao et al., 2002) in Taiwan concluded that there was no significant differences with SEN V infection in patients with liver disease than in patients without liver disease, liver function, age, gender, amount of alcohol intake, and history of blood transfusion between SEN-V-positive and SEN-V-negative, so in many investigation of disease associations with SENV infection should not be limited to hepatitisinfection. It may consider that virus may not be

a pathogenicor may be opportunistic under special circumstances like an acquired immunodeficiency

Conclusion

The results of the recent study indicated that 30% of healthy blood donors Iraqi participants have been infected with SEN V-D and SEN V-H in both genders. The SEN V-H was the predominant genotype and more related to Asian strains.

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