JJBS

Jordan Journal of Biological Sciences

Volume 12, Number 3,August 2019
ISSN 1995-6673
Pages 355 - 360

Detection and Genotyping of SEN Virus among Patients with Hepatitis and Healthy Blood Donors from Baghdad, Iraq

Ealaf A. Khudair [1], Arwa M. Abdullah Al-Shuwaikh [1*] and Nawal M. Farhan [2]

[1]Microbiology Department, College of Medicine, Al-Nahrain University, Al-Kadhimiya, P.O. Box 70098 ; [2]Gastroenetrology and Hepatology Teaching Hospital, Baghdad – Iraq

Received August 20, 2018; Revised October 17 5, 2018; Accepted November 8, 2018

Abstract

SEN virus (SENV) was discovered in 1999 as a DNA virus with hepatotropic properties. This study aims at determining the prevalence of SENV infection and genotypic characteristics in hepatitis patients and healthy blood donors. Serum samples were collected from fifty patients with a history of hepatitis B or C. In addition, fifty sera were collected from healthy blood donors as a control group. The serum samples were tested by nested polymerase chain reaction (PCR) for the detection of SENV DNA and its two genotypes (SENV-H and SENV-D). SENV was detected in 42.0 % (twenty-one out of fifty) of the hepatitis patients compared to 20 % (ten out of fifty) of the control group. SENV-H was detected in a higher prevalence than SENV-D among cases and the control group; seventeen (81 %) and nine (90 %), respectively, among cases and four (19 %) and one (10 %), respectively, among the blood donors in the control group. The prevalence of SENV in hepatitis patients was significantly higher than in healthy blood donors. There was no statistically significant relationship between SENV positivity and the mean level of liver enzyme. Hence, infection with SENV was not associated with the increased severity of the liver diseases even among HBV or HCV positive patients.

Keywords: SENV, Hepatitis B virus, Hepatitis C virus, Healthy blood donors, Prevalence, Genotype.

1. Introduction

The relatively recently discovered DNA virus SEN virus (SENV) was suspected to be significantly associated with hepatitis (Yoshida *et al.*, 2002). It was proposed that SENV belongs to a new virus family named *Anelloviridae* (Sagir*et al.*, 2004).

SENV has been described as a blood-borne pathogen that has a worldwide incidence (Sagir*et al.*, 2004). A wide range of SENV infections are reported in individuals who have a liver disease or who are human immunodeficiency (HIV)- positive, or in intravenous drug users, thalassemic patients, and patients on maintenance hemodialysis (Karimi-rastehkenari and Bouzari, 2010). SENV was previously detected in 67 % and 41 % of patients with the Hepatitis C virus (HCV) and the Hepatitis B virus (HBV), respectively. SENV was also detected in 16 % of the healthy blood donors (Kao *et al.*, 2003; El-hady *et al.*, 2006).

About 20 % of hepatic infections are not associated with hepatitis viruses (A–E) and might be attributed to other viruses. There is a very strong association between two strains of SENV (SENV-H and SENV-D) and the development of a non-A to E hepatitis infection. SENV is considered as a post-transfusion hepatitis virus. However, because the majority of SENV-infected patients do not develop hepatitis, causality is difficult to establish (Hosseini and Bouzari, 2016). Although the pathogenicity of SENV is not fully clear, SENV can undoubtedly infect patients who are already infected with other viruses. The most important high-risk persons are those infected with HBV and HCV (Kao *et al.*, 2003; Dehkordi and Doosti, 2011). A previous study indicated that SENV has a positive impact on liver pathology by decreasing liver damage, which in turn can result in a reduction of liver enzyme levels (Hosseini and Bouzari, 2016).

This study aims at determining the frequency of SENV viremia and the genotypes (SENV-D and SENV-H) by performing nested-PCR in patients with HBV, HCV or healthy blood donors, and to estimate the level of liver enzymes and risk factors.

2. Materials and Methods

2.1. Subjects

This study has a case control design. A total of one-hundred blood samples were collected from fifty patients with HBV or HCV from the Gastroenterology and Hepatology Teaching Hospital over the period from November, 2017 to March, 2018. Another fifty blood samples were collected from healthy blood donors at the Blood Donation Center in Al Imamein Al Kadhimein Medical City. The clinical characteristics of both patients and healthy individuals in the control such as (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), HCV-Ab, HBsAg and HBcAb) were obtained from medical records. The study has been approved by the Ethical Committee of Al-Nahrain Collage of Medicine.

* Corresponding author e-mail: arwa.mujahid@colmed-alnahrain.edu.iq; arwa_alshwaikh_2004@yahoo.com.

2.2. Specimens' Collection:

The serum samples were collected from all patients and individuals in the control group by venipuncture of the median cubital vein. Five mL blood samples were collected in sterile gel tubes without any anticoagulant. The samples were allowed to clot at the room temperature within one hour of collection, before centrifugation at 3,000 rpm for ten minutes, and were then stored frozen at (-44) ˚C until testing.

2.3. DNA Extraction:

The serum samples were removed from the deep freezer (-44) and were allowed to thaw at the room temperature. Viral Nucleic Acid Extraction Kit II (Cat. #VR00, Geneaid, Taiwan) was used for the isolation and purification of DNA from the samples. The procedure was done according to the manufacturer's instructions.

2.4. SEN virus DNA Amplification:

DNA amplification reactions were carried out by the nested conventional PCR according to (Hosseini and Bouzari, 2016) with modifications to optimize the results using first round primers SENV-AI-1F (TWCYCM-AACGACCAGCTAGACCT) and SENV-AI-1R (GTTTGTGGTGAGCAGAACGGA) (Alpha DNA, USA) for the SENV detection, while second round primers were SENV-D-1148F (TTTGGCTGCACCTTCTGGTT) and SENV-D-1341R (AGAAATGATGGGTGAGTGTTAGG-G) (Alpha DNA, USA) for the SENV-D genotype detection and SENV-H-1020F (CTAAGCAGCCCTAAC-ACTCATCCAG) and SENV-H-1138R (GCAGTTGA-CCGCAAAGTTACAAGAG) (Alpha DNA, USA) for the SENV-H genotype detection. For the first round reaction, the following components were mixed together in AccuPower® ProFi Taq PCR PreMix tube (Bioneer Korea): 2 µL of 10 uM/µL (SENV-AI-1F) primer and 2 µL of 10 uM/µL (SENV-AI-1R) primer, 3 µL of DNA and 13 µL of DNase free sterile water (Promega, USA), in a final reaction volume of 20 uL without calculating the volume of the lyophilized pellet of the AccuPower® PCR PreMix tubes. For the second-round reaction, the following components were mixed together in AccuPower® ProFi Taq PCR PreMix tube (Bioneer Korea): 1 µL of 10 uM/µL (SENV-D-1148F) primer and 1 µL of 10 uM/µL (SENV-D-1341R) primer or 1 µL of 10 uM/µL (SENV-H-1020F) primer and 1 µL of 10 uM/µL (SENV-H-1138R) primer, 1 µL of amplified DNA from the first PCR run, 17 µL of DNase free sterile water (Promega, USA), in a final reaction volume of 20 µL (without taking into account the volume of the lyophilized pellet of the AccuPower® PCR PreMix tubes). Cycling conditions for both the first- and second-round reactions were as follows: initial denaturation 95˚C for five minutes (1 cycle), DNA amplification by sequential denaturation of DNA at 95˚C for thirty sceonds, annealing at 60˚C for forty-five seconds, and extension at 72˚C for forty-five seconds (thirty-five cycles), and a final extension at 72˚C for five minutes (one cycle) as shown in Table 1. For visualization, the PCR amplification products were subjected to electrophoresis on 1 % agarose (Bio Basic, Canada) in 1X TBE solution (Promega, USA). The SENV-DNA positive samples showed a 349 bp band for all SENV genotypes after the first PCR round, and a 124 bp band for SENV-H or 198 bp band for SENV-D after the second PCR round (Hosseini and Bouzari, 2016). Positive control for SENV DNA was chosen after a random screening of the serum samples and confirmation by sequencing, while the negative control consisted of a reaction tube without a template DNA. Positive and negative control groups were run with each reaction.

Table 1. PCR program for both first and second round reaction.

Step	Temperature (ºC)	Time	Cycle
1	95	5 min	1
2	95	30 s	35
	60	45 s	
	72	45 s	
3	72	5 min	1

2.5. Statistical Analysis

Analysis of data was carried out using the Statistical Package for Social Sciences (SPSS) (version 19). Categorical data were presented as count and percentage, and the differences were examined by Chi-square test (X^2-test) or Fisher's exact test. On the other hand, numerical data were presented as mean ± standard deviation (SD), and evaluated by the independent sample T-test. Statistical significance was considered at a *P* value equal or less than 0.05.

3. Results

This study includes fifty hepatitis patients with HBV or HCV infection, with a mean age of 36.20 ± 13.4 years, in addition to fifty healthy blood donors as control with a mean age of 35.22 ± 9.8 years. Thirteen out of fifty (26 %) patients were hepatitis C-positive, while thirty-seven out of fifty (74 %) patients were hepatitis B- positive. Regarding gender distribution, there were twenty-four (48 %) males and twenty-six (52 %) females among the patients' group, compared to forty-seven (94%) males and three (6%) females among the control group. The mean values of the liver function test parameters i.e., ALT and AST, were higher among hepatitis patients than in healthy blood donors [50.9 ± 49.7 vs. 13.46 ± 3.840 (U/l)] and [52.6 ± 56.5 vs. 24.58 ± 7.271 (U/l)], respectively, as shown in Table 2.

Table 2. Distribution of patients and control according to age, gender and type of hepatitis infection.

Variables		Category: Patients group (n= 50)	Category: Controls group (n= 50)	Statistic
Age (mean ± SD) year		36.20 ± 13.4	35.22 ± 9.8	*P*= 0.679*
Gender	Male (n= 71)	24 (48.0 %)	47 (94.0 %)	*P*= 0.000**
	Female (n= 29)	26 (52.0 %)	3 (6.0 %)	
ALT (mean ± SD) (U/l) ALT (U/l)		50.9 ±49.7	13 .46 ±3.840	*P* =0.000*
AST (mean ± SD) (U/l)		52.6 ±56.5	24.58 ±7.271	*P* =0.001*
Type of Hepatitis	HBV	37 (74.0 %)	None	----------
	HCV	13 (26.0 %)	None	

* Using T-test at 0.05 level.
** Using Chi-square test at 0.05 level.
*** Normal values: AST 15-37 U/L, ALT 12-78 U/L

3.1. Detection of SEN Virus

SENV DNA was detected in twenty-one out of fifty (42.0 %) patients by the nested conventional PCR, while only ten out of fifty (20 %) in the control group were found to be SENV-DNA positive. The patients' group was 2.897 times more likely to be SENV-DNA positive as the control group [Odds Ratio (OR) = 2.897] as shown in Table 3 and Figure 1.

Table 3. Frequency of SENV DNA among cases and controls.

SENV DNA status	Category: Patients group (n = 50)	Category: Controls group (n = 50)	Total (%)	Statistic*
No. of positive (%)	21 (42.0)	10 (20.0)	31 (31)	X^2 = 5.657
No. of negative (%)	29 (58.0)	40 (80.0)	69 (69)	P = 0.017
Total (%)	50 (100.0)	50 (100.0)	100 (100)	

*(OR= 2.897; 95% CI, 1.19-7.07)

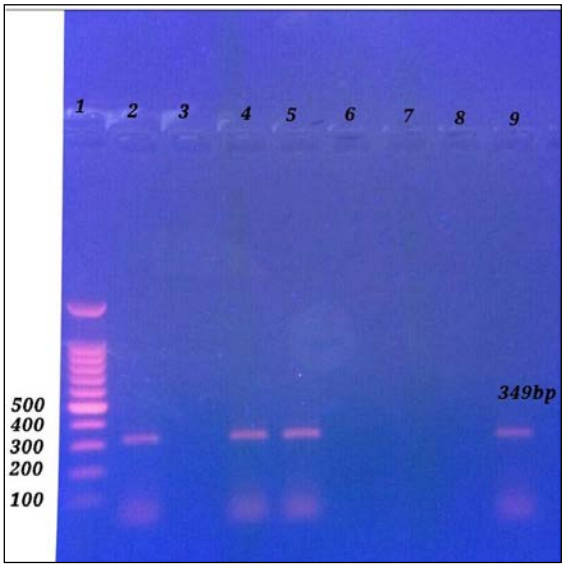

Figure 1. Gel-electrophoresis of first round PCR products on 1% agarose. Lane1, 100bp DNA marker, Lane2: positive control, Lane3: negative control, Lanes 4,5, and 9: positive samples, Lanes 6,7, and 8: negative samples.

3.2. SEN Virus Genotyping

SENV-H was detected in 81.0 % of SENV-positive patients (seventeen out of twenty-one patients; eleven HBV patients and six HCV patients) and in 90.0 % of the SENV-positive blood donors in the control. SENV-D was detected in 19.0 % of SENV- positive patients (four out of twenty-one patients; three HBV patients and one HCV patient), and in 10.0 % of the SENV-positive blood donors in the control group, as shown in Table 4 and Figures 2 and 3). In all of the studied groups, the frequency of SENV-H was higher than SENV-D. However, this difference did not reach the level of statistical significance (P>0.05).

Table 4. Detection of SENV-H and SENV-D genotype in SENV positive cases.

SENV Genotypes	Patients group (n = 50)	Controls group (n = 50)	Total (%)	Statistic
No. of SENV-H (%)	17 (81.0)	9 (90.0)	26 (83.9)	
No. of SENV-D (%)	4 (19.0)	1 (10.0)	5 (16.1)	P= 0.522
Total (%)	21 (100.0)	10 (100.0)	31(100.0)	

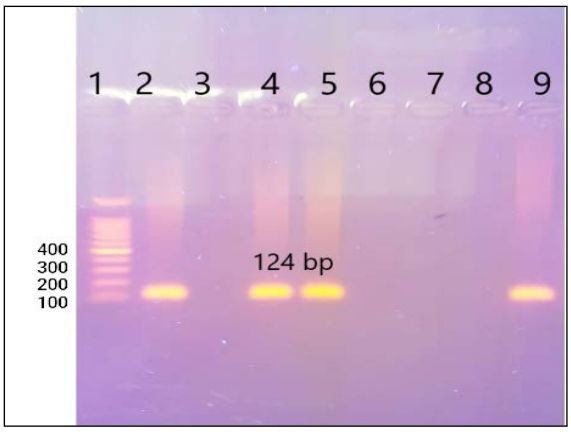

Figure 2. Gel-electrophoresis of second-round PCR products for SENV-H genotype using 1 % agarose. Lane1, 100bp DNA marker, Lane 2: positive control, Lane 3: negative control, Lanes 4, 5, and 9: positive samples, Lanes 6, 7, and 8: negative samples.

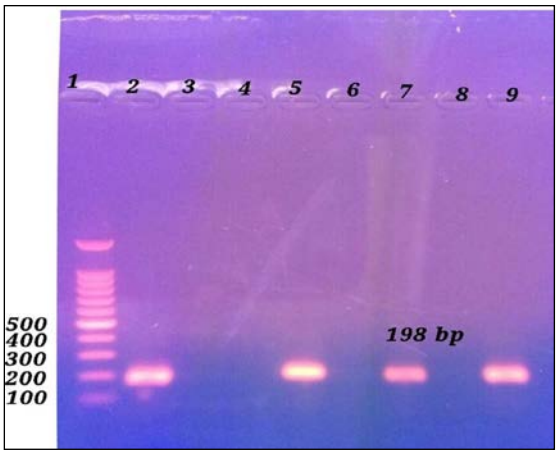

Figure 3. Gel-electrophoresis of the second-round PCR products for SENV-D genotype using 1 % agarose in TBE buffer. Lane 1, 100bp DNA marker, Lane 2: positive control for amplification, Lane 3: negative control, Lane 5, 7, 9: positive samples, Lane 4, 6, 8: negative samples.

3.3. Liver Enzymes

This study shows that the biochemical parameters i.e., liver enzymes (ALT and AST) did not significantly differ between SENV-positive and SENV-negative individuals among each of the study groups, as shown in Table 5.

Table 5. Serum ALT and AST level (U/L) in relation to SENV DNA status in patients and control groups.

Biochemical Test*	Patients group (n=50)		Statistic	Controls group (n=50)		Statistic
	SENV +Ve	SENV -Ve		SENV +Ve	SENV -Ve	
ALT (U/l)	52.48 ± 49.483	49.83 ± 50.851	t= -0.184 *P*= 0.855	14.40 ± 4.142	13.23 ± 3.779	t= -8.63 *P*= 0.931
AST (U/l)	53.62 ± 56.662	52.00 ± 57.498	t= -0.99 *P*= 0.922	29.60 ± 8.972	25.0 ± 3.80	t= -1.282 *P*= 0.230

*Values are presented as mean ± SD, Normal values: ALT (12-78) U/L and AST (15-37) U/L

3.4. Risk Factors

Concerning risk factors for individuals in this study, there was no statistically significant difference in the mean age between SENV-positive and SENV-negative individuals. Furthermore, gender, history of blood transfusion, tattooing, and surgery, did not affect the prevalence of SENV (*P*>0.05), Table 6.

Table 6. The association between SENV infection and the risk factors.

Risk factors		SENV		Total	Statistic
		Positive	Negative		
Mean of age ± SD		34.94 ± 10.692	36.06 ± 12.253	35.71 ± 11.749	t= 0.440 *P*= 0.661
Gender	Male (%)	23 (74.2)	48 (69.6)	71 (71.0)	X^2= 0.637 *P*= 0.223
	Female (%)	8 (25.8)	21 (30.4)	29 (29.0)	
	Total (%)	31 (100)	69 (100)	100 (100)	
History of Blood Transfusion	Presence (%)	2 (6.5)	2 (2.9)	4 (4.0)	X^2= 0.703 *P*= 0.402
	Absence (%)	29 (93.5)	67 (97.1)	96 (96.0)	
	Total (%)	31 (100.0)	69(100.0)	100 (100)	
History of Surgery	Presence (%)	10(32.3)	21 (30.4)	31(31.0)	X^2= 0.033 *P*= 0.855
	Absence (%)	21 (67.7)	48 (69.6)	69(69.0)	
	Total (%)	31 (100.0)	69(100.0)	100 (100)	
History of Tattooing	Presence (%)	6(19.4)	8(11.6)	14(14.0)	X^2= 1.070 *P*= 0.301
	Absence (%)	25 (80.6)	61(88.4)	86(86.0)	
	Total (%)	31(100.0)	69(100.0)	100 (100)	

4. Discussion

4.1. Detection and Genotyping of SEN Virus:

In the present study, SENV was detected in a considerable percentage of hepatitis patients with genotype H being the most prevalent. The prevalence of SENV infection was 42.0 % in the hepatitis patients and 20 % in the healthy blood donors of the control group. The SENV-prevalence rate among HCV patients is in agreement with the average prevalence rates reported in Egypt which ranged from 13.5 % to 49 % (Elsherbiny *et al.*, 2015). Globally, SENV prevalence rate was around 21 % reaching up to 69 % *(Yoshida et al., 2002; Wong et al.*, 2002). Also, results of this study are similar to those reported by Kao *et al*. (2003) who reported a high prevalence (41 %) of SENV-infection among patients with HBV. The results of Mu *et al.*(2004) showed that the prevalence rate of SENV-infection in patients with HBV was 59 % (Mu *et al.*, 2004). In this study, the percentage of SENV in the control group was 20 %. This is consistent with the range reported from other countries such as Japan (10 % to 22%) (Shibata *et al.*, 2001), Germany (8 % to 17 %) (Schröter *et al.*, 2002), Taiwan (15 %) (Kao*et al*., 2003), Italy (13 %) (Pirovano *et al.*, 2002), and Egypt (16 % to 20 %) (Mohamed *et al.*, 2011). In contrast, the results of the current study are much lower than those reported in Japan (75 %) and Isfahan (90.8 %; a central province in Iran) (Karimi-rastehkenari and Bouzari, 2010; Gerner and Wirth, 2002).

The prevalence of SENV-D/H DNA was 42.0 %. The distribution of SENV-D and SENV-H infections slightly varied between the hepatitis patients group (81.0 % for SENV-H, 19.0 % for SENV-D) and the control group (90.0 % for SENV-H, 10.0 % for SENV-D). The frequency of SENV-H was higher compared to SENV-D in both groups. However, the difference between the two groups was not statistically significant (*P*>0.05). These results were similar to those reported from Turkey and Taiwan (Serin *et al.*, 2006 and Kao *et al.*, 2002), as well as Iran (Karimi-rastehkenari and Bouzari, 2010), but are different from those reported in Egypt in which SENV-D was detected in all SENV-positive samples of the control group (Mohamed *et al.*, 2011), and from Japan which demonstrated SENV-D in 77 % and SENV-H in 15 %, of the healthy individuals (Kobayashi *et al.*, 2003).

On the whole, the SENV viremia rate differed from that reported by others (Dehkordi and Doosti, 2011; Hosseini and Bouzari, 2016; Abbasi*et al.*, 2019). The variability in SENV prevalence across different geographical regions of the world is attributed to the differences in the methods used and interactions among biological, behavioral, and social factors (El-hady *et al*., 2006). Other reasons behind this variability include differences in the quantity of SENV DNA in the sera, the use of different target sequences; un-translated region vs. open reading frame (UTR vs. ORF), differences in the sensitivities of the assay systems used or other reasons such as intravenous drug use, unsafe sexual practices, homosexuality, and professional exposure (Yoshida*et al.*, 2002).

4.2. Liver Enzymes and Risk Factors Association with SENV.

No significant relation was observed between the level of either ALT or AST or both, in the hepatitis patient or control groups, with a SENV-infection status, as shown in Table (5), suggesting that the presence of SENV did not cause an increase in the severity of liver damage in the patient group. This is similar to a pervious study from Egypt that showed a statistically insignificant difference in SENV viremia between HCV patients and HCV-related hepatocellular carcinoma patients (Kholeif and Fayez, 2008). Furthermore, Schröteret *et al.* (2002), Sagir *et al.* (2004), and Borawski *et al.* (2006) did not observe any effects on the liver enzyme levels (ALT and AST) due to the SENV infection among the hepatitis patients. In contrast, another study maintained that the levels of liver enzymes were significantly lower in the HBV patients co-infected with SENV compared to the HBV patients, indicating a positive impact of the virus on liver pathology by decreasing liver damage, and thus decreasing serum liver enzyme levels (Hosseini and Bouzari, 2016).

Most of the SEN-virus-positive individuals had a mean age of 34.94 ± 10.692 years. However, there was no significant difference in age between SENV-positive and SENV-negative among the hepatitis patients and the healthy blood donors. This is in agreement with another study that reported no significant difference between SENV-positive and SENV-negative liver patients according to age (Kholeif and Fayez, 2008). Another study reported a high prevalence of SENV among younger ages (Chiou *et al.*, 2006). No significant association with age was found in the present study.

SENV infection was found at higher proportions among males than females (74.2 % vs. 25.8 %). In spite of this, the difference was not statistically significant (P>0.05) suggesting that males and females have the same chance for SENV infection. This is in agreement with a study by Loutfy *et al*., (2009) who detected no significant differences in age and gender between SENV-positive and SENV-negative hepatitis patients. In contrast, Chiou *et al*., (2006), Kobayashi *et al.*, (2003), and Schréter *et al*., (2006) described a notable difference in the SENV prevalence according to gender with a higher proportion among the males of the SENV-positive patients**.**

The present study did not report any significant association between SENV infections and the history of blood transfusion; this may be due to the limited number of individuals having a history of blood transfusion in this study (four out of one-hundred). In addition, other studies reported that SENV was not associated with blood transfusion history (Yoshida*et al.*, 2002; Tang *et al.*, 2008) indicating that blood transfusion transmission is not the only way for people to be infected with SENV (Karimi-rastehkenari and Bouzari, 2010; Tang *et al.*, 2008). However, a previous study conducted by Mohamed *et al.* (2011) in Egypt, showed a significant difference between SENV-positive and SENV-negative patients regarding blood transfusions. The fact that SENV is also observed in healthy blood donors who had no history of blood transfusions suggests that it could be transmitted through other means than blood and injection. Possible mechanisms of transmission include fecal-oral route, saliva, amniotic fluid from SENV-positive women, breast milk, bile, and other tissues (Okamoto *et al.*, 2000).

The current study showed no significant association between SENV positivity and individuals with history of surgery. Interestingly, another study indicated that 3 % of patients who underwent an open-heart surgery were acutely infected with SENV in a complete absence of blood transfusion which suggests a nosocomial transmission of the virus as a consequence of using contaminated fomites and intravascular catheters, as well as materials associated with postoperative wounds including elastic bandages for surgical wounds. Also, instruments, equipment, and wound -dressing material may act as sources of infection as a result of contamination from blood and blood products (Umemura*et al.*, 2001).

The results of the epidemiologic studies regarding the risk of viral infections among tattooed individuals are conflicting (Deschesnes *et al.*, 2006; Jafari *et al.*, 2012). Therefore, this study also tried to investigate the relationship between SENV infection and tattooing in order to determine the risk of transmission of SENV infection. The present study didn't find an association between the history of tattooing and SENV infection in both of the study groups. These findings are consistent with a previous study from Slovaka (Schreter and Jarcuska, 2006).

In conclusion, the current study found that SENV did not seem to contribute to the pathogenesis of liver diseases among HBV- or HCV-infected patients. Further studies are still required with large samples of patients and controls, coupled with viral load quantification, to estimate the risk factors, mode of transmission and pathogenesis of SENV.

Acknowledgment

Sincere thanks go to the staff members in Blood Donation Center in Al-Imammain Al-kadhimain Medical City Hospital and Gastroenterology and Hepatology Teaching Hospital for their assistance in the sample collection. Dr. Arwa Mujahid Al-Shuwaikh received funding from L'Oreal-UNESCO for Women in Science Levant and Egypt.

Author contributions

All authors contributed to this manuscript by one way or another. Dr. Arwa M. Al-Shuwaikh designed, interpreted, and arranged this manuscript. Ealaf A. Khudair performed all the laboratory work and implementation of this study, and Dr. Nawal M. Farhan helped in the clinical aspects and the collection of the samples.

Conflict of Interest

There is no conflict of interest.

Funding

This project was partially supported by L'Oreal-UNESCO for Women in Science.

References

Abbasi S, Makvandi M, Karimi G and Neisi N. 2019. The prevalence of SEN virus and occult hepatitis B (OBI) virus infection among blood donors in Ahvaz City. *Jundishapur J Microbiol*, **(7):** 3-8.

Borawski J, Kovalchuk O, Chlebińska I, Naumnik B, Rydzewska-Rosołowska A, Chyczewski L, *et al.* 2006. SEN virus infection in maintenance hemodialysis patients. *Wiad Lek*, **59(11–12):** 751-756.

Chiou SS, Huang JF, Chang TT, Hsieh MY, Dai CY, Yu ML, *et al.* 2006. SEN and hepatitis virus infections in nontransfused children and pediatric thalassemia patients with multiple transfusions in Taiwan. *Digestion*, **74(3–4):** 208-214.

Dehkordi PG and Doosti A. 2011. The prevalence of SEN virus infection in blood donors and chronic hepatitis B and C patients in Chaharmahal Va Bakhtiari province. *J. Cell Anim. Biol*, **5(8):** 182-186.

Deschesnes M, Demers S and Finès P. 2006. Prevalence and Characteristics of Body Piercing and Tattooing Among High School Students. *Can J Public Heal / Rev Can Sante'e Publique*, **97(4):** 325-329.

El-Hady SIA, Zaki SMI, El-Sayed AA, El-din MAE, Nadia M and Salamouny E-. 2006. Prevalence of SEN Virus (SEN-V) Infection

among patients on maintenance hemodialysis in Egypt. *Egyptian Journal of Medical Microbiology*, **15(1):** 99-112.

Elsherbiny NM, Hassan EA, Ahmed AO, Abd El-RehimAS, Abd *El* Rehim N and Hassany SM. 2015. Does SEN virus (SEN-V) infection affect the progression of chronic hepatitis C or B among Egyptian patients ? *Afr. J. Microbiol. Res*, **9(23):** 1504-1512.

Gerner P and Wirth S. 2002. Eigenschaften und klinische Bedeutung neuer hepatotroper Viren Hepatitis-G-Virus, TT-Virus und SEN-Virus. *Monatsschrift Kinderheilkd*, **150(1):** 19-26.

Hosseini SA and Bouzari M. 2016. Detection of SENV virus in healthy, hepatitis B- and hepatitis C-infected individuals in Yazd Province, Iran. *Iran Biomed J*, **20(3):** 168-174.

Jafari S, Buxton JA, Afshar K, Copes R and Baharlou S. 2012. Tattooing and Risk of Hepatitis B: A Systematic Review and Meta-analysis. *Can J Public Heal / Rev Can Sante'e Publique*. **103(3):** 207-212.

Kao JH, Chen W, Chen PJ, Lai MY and Chen DS. 2003. SEN virus infection in patients with chronic hepatitis C: preferential coinfection with hepatitis C genotype 2a and no effect on response to therapy with interferon plus ribavirin. *J Infect Dis*, **187(2):** 307-310.

Kao JH, Chen W, Chen PJ, Lai MYand Chen DS. 2002. Prevalence and implication of a newly identified infectious agent (SEN virus) in Taiwan. *J Infect Dis*., **185(3):** 389-392.

Karimi-rastehkenari A and Bouzari M. 2010. High frequency of SEN virus infection in thalassemic patients and healthy blood donors in Iran. *Virol J*, **7:** 1.

Kholeif L and Fayez D. 2008. SEN Virus Detection in Egyptian Patients with Chronic Hepatitis C and Hepatitis C Related Hepatocellular Carcinoma. *Egyptian Journal of Medical Microbiology*, **17(1):** 91-97.

Kobayashi N, Tanaka E, Umemura T, Matsumoto A, Iijima T, Higuchi M, Hora K and Kiyosawa K. 2003. Clinical significance of SEN virus infection in patients on maintenance haemodialysis. *Nephrol Dial Transplant*; **18(2):** 348-352.

Loutfy SA, Hafez MM, Massoud WA, Fotuh NA, Moneer MM and Zaghloul HS. 2009. SEN virus infection in Egyptian patients undergoing maintenance hemodialysis: Prevalence and clinical importance. *J Microbiol Immunol Infect*, **42(6):** 464-470.

Mohamed S, Thabit AG, Abd-El Rahman SA, Abdelmohsen EEA, Seif Eldin SS and Ghandour AMA. 2011. Prevalence of SEN virus infection in multi transfused patients in Assiut University Hospitals, Egypt. *Journal of American sciences*, **7(1):** 687-696.

Mu S-J, Du J, Zhan L-S, Wang H-P, Chen R, Wang Q-L, *et al.* 2004. Prevalence of a newly identified SEN virus in China. *World J Gastroenterol*, **10(16):** 2402-2405.

Okamoto H, Nishizawa T, Tawara A, Takahashi M, Kishimoto J, Sai T, *et al*. 2000. TT Virus mRNAs Detected in the Bone Marrow Cells from an Infected Individual. *Biochem Biophys Res Commun*, **279(2):** 700-707.

Pirovano S, Bellinzoni M, Ballerini C, Cariani E, Duse M, Albertini A, *et al.* 2002. Transmission of SEN virus from mothers to their babies. *J Med Virol*, **66(3):** 421-427.

Sagir A, Adams O, Kirschberg O, Erhardt A, Heintges T and Häussinger D. 2004. SEN virus does not affect treatment response in hepatitis C virus coinfected patients but SEN virus response depends on SEN virus DNA concentration. *World J Gastroenterol*, **10(13):** 1893-1897.

Schreter I and Jarcuska P. 2006. Detection of SEN Virus in the General Population and Different Risk Groups in Slovakia. *Folia Microbiologica*, **51 (3)**, 223-228.

Schröter M, Laufs R, Zöllner B, Knödler B, Schäfer P, Sterneck M, *et al*. 2002. Prevalence of SENV-H viraemia among healthy subjects and individuals at risk for parenterally transmitted diseases in Germany. *J Viral Hepat*, **9(6):** 455-459.

Serin MS, Tezcan S, Delialioglu N, Tiftik N, Aslan G and Emekdas G. 2006. Investigation of SEN virus genotypes D and H among blood donors in Mersin University Medical School Hospital. *Mikrobiyoloji Bülteni*, **40(1-2):** 39-45.

Shibata M, Wang RY, Yoshiba M, Shih JW, Alter HJ and Mitamura K. 2001. The presence of a newly identified infectious agent (SEN virus) in patients with liver diseases and in blood donors in Japan. *J Infect Dis*, **184(4):** 400-404.

Tang ZH, Chen XH, Yu YS and Zang GQ. 2008. Prevalence and clinical significance of SEN virus infection in patients with non A-E hepatitis and volunteer blood donors in Shanghai. *World J Gastroenterol*. **14(26):** 4204-4208.

Umemura T, Alter HJ, Tanaka E, Yeo AE, Shih JW, Orii K, *et al.* 2001. Association between SEN virus infection and hepatitis C in Japan. *J Infect Dis*, **184(10):** 1246-1251.

Wong SG, Primi D, Kojima H, Sottini A, Giulivi A, Zhang M, *et al.* 2002. Insights into SEN virus prevalence, transmission, and treatment in community-based persons and patients with liver disease referred to a liver disease unit. *Clin Infect Dis*, **35(7):** 789-795.

Yoshida H, Kato N, Shiratori Y, Shao R, Wang Y, Shiina S, *et al.* 2002. Weak association between SEN virus viremia and liver disease. *J Clin Microbiol*, **40(9):** 3140-3145.