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Full Length Research Paper

Does *SEN* virus (*SENV*) infection affect the progression of chronic hepatitis C or B among Egyptian patients?

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The effect of *SENV* infection on chronic viral hepatitis is not very clear till now. Information regarding *SENV* infections in the Egyptian population where hepatitis viruses are prevalent is limited to a certain extent. We aimed to determine the frequency of *SENV* and its genotypes H and D in Egyptian patients with chronic hepatitis B (CHB) or C (CHC) and to study its possible role in the progression of liver disease. A total of 112 patients with chronic hepatitis (18 CHB and 94 CHC) were subjected to clinical assessment, laboratory and histological examinations. DNA from sera was extracted and *SENV* DNA was amplified by polymerase chain reaction. *SENV* DNA was detected in 28.6% of patients with chronic hepatitis (32/112). The percentage was 33.3 and 27.7% in CHB and CHC patients respectively. *SENV-H* was detected more frequently than *SENV-D* genotype. For CHC patients without cirrhosis (n=44), *SENV* was negative in 90.9% (40/44) compared to 9.1% (4/44) *SENV* positive giving a statistically significant difference (<0.001). A significantly higher percentage of patients showed twofold or more increase in the aspartate transaminase (AST) and alkaline phosphatase (ALP) among *SENV* negative CHC patients compared to *SENV* positive patients (P=0.04 , 0.03 respectively). In cirrhotic patients (n=58), both the levels of ALP and serum total bilirubin were significantly higher in *SENV* negative compared to *SENV* positive patients (P values were 0.01). For CHB patients, no statistically significant difference was detected regarding any of the studied parameters. We conclude that *SENV* does not worsen the progression of chronic viral hepatitis. This may reflect a possible protective effect of *SENV* in CHC patients which needs to be emphasized by further larger studies.

Key words: *SENV*, chronic, viral, hepatitis.

INTRODUCTION

The *SEN* virus (*SENV*) was considered to be a member of the family *Circoviridae*, genus Anellovirus, a group of non- enveloped, circular DNA viruses that also included the Torque teno (TTV) and its variants SANBAN,

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YONBAN, TUS01, and PMV (Sugiura et al., 2004). Now *Anelloviridae* is a highly divergent family of viruses that has three genera of anelloviruses capable of infecting humans: torque teno virus (TTV; *Alphatorquevirus*), torque teno minivirus (TTMV; *Betatorquevirus*), and torque teno midivirus (TTMDV; *Gammatorquevirus*) (Biagini, 2009).

SENV has a different geographic distribution and is fairly common around the world. Its prevalence has been found to vary in different populations (Tezcan et al., 2009). Phylogenetic analysis of *SENV* has demonstrated nine different genotypes: *SENV-A* to *SENV-I* which show 15-50% sequence diversities among them (Kojima et al., 2003). *SENV-H* and *SENV-D* genotypes were extremely associated with non-A to E hepatitis (Schroter et al., 2003).

Although not all transmission routes have been identified (Tezcan et al., 2009), yet it was reported that *SENV-D/H* could be transmitted by both parenteral and non-parenteral routes (faecal oral) (Umemura et al., 2001). Iatrogenic means in hospital setting is reported as a mode of transmission (Sagir et al., 1994). In addition, vertical transmission from mother to fetus does occur (Pirovano et al., 2002).

The prevalence of *SENV* has been investigated in patients with various forms of liver disease in many countries. *SENV* infection is frequently observed in patients with hepatitis B virus (HBV) (23 to 59%), hepatitis C virus (HCV) (22 to 89%), and in patients with hepatitis of unknown etiology (Schreter et al., 2006).

SENV may cause persistant infection that may exceed one year and has been documented as long as 12 years (Umemura et al., 2002). The role of *SENV* infection and the clinical significance were studied in patients with non A-E hepatitis or other viral hepatitis but the results are not very clear and are inconsistent to some extent and even show contradictory results (Wang et al., 2007; Cakaloglu et al., 2008). That is to say, coinfection with hepatitis B virus (HBV), or hepatitis C virus (HCV) has been reported to be associated with severe and progressive liver disease (Jardi et al., 2001). However, others reported that *SENV* was found to have no established pathogenicity and the exact role of this virus in the pathogenesis of liver diseases, including acute and chronic hepatitis, cirrhosis and the development of hepatocellular carcinoma (HCC) remains to be verified (Rizvi et al., 2013). Others suggested a protective role of *SENV* against HCV (Umemura et al., 2001). Information regarding *SENV* infections in the Egyptian population where HBV and HCV are prevalent is limited to a certain extent.

It has been stated that Egypt has the highest prevalence of hepatitis C worldwide and the epidemic will soon peak (Yahia, 2011). Overall, HCV prevalence among blood donors ranged between 5-25%, and among other general population groups between 0-40%. HCV prevalence among multi-transfused patients ranged between 10-55%, and among high risk populations reached up to 85% (Mohamoud et al., 2013). For HBV, the prevalence in Egypt was found to be 5% (Awadalla et al., 2011).

The aim of present study was to determine the frequency of *SENV* and its genotypes H and D in Egyptian patients with chronic hepatitis B (CHB) or C (CHC) and to study its possible role in the progression of liver disease in such patients.

MATERIALS AND METHODS

Study design

This cross sectional study was done in Assiut University Hospital after being approved by the Ethical Committee of the Faculty of Medicine, Assiut University. A verbal consent was also taken from the patients enrolled in the study.

Patients

A total of 112 patients with CHC or CHB were included in the study during a six months period (from June to December 2013). Patients were admitted to the department of Tropical Medicine and Gastroenterology, Assiut University. They were divided into 2 groups; patients with CHC (n=94) and CHB (n=18). None of the patients had a history of receiving antiviral therapy. For all patients, thorough clinical assessment, abdominal ultrasonography, laboratory tests and liver histopathology were done. The severity of cirrhosis was assessed by Child-Pugh classification (Child and Turcotte, 1964; Pugh et al., 1973)

We defined chronic hepatitis as persistent or intermittent elevation in alanine transaminase (ALT) or aspartate transaminase (AST) levels for more than 6 months with the presence of anti-HCV antibodies and positive serum HCV RNA for chronic hepatitis C (Anwar et al., 2006), positive HBsAg and positive serum HBV DNA for chronic hepatitis B and by liver biopsy showing chronic hepatitis with moderate or severe necroinflammation (Huntzinger, 2009).

Healthy controls

The control group included 20 healthy individuals of comparable age among which 12 (60%) were men and 8 (40%) women; their mean age being 45 years ± 3.6. These individuals were negative for HBsAg and anti-HCV.

Laboratory tests

Five milliliters blood was withdrawn from each subject under complete aseptic conditions. Sera were separated and stored frozen at -20°C until analysis. For all serum samples, the following laboratory tests were performed: Liver function tests (aspartate-aminotransferase (AST), alanine transaminase (ALT), Alkaline phosphatase (ALP), albumin, bilirubin and prothrombin time); Serological tests for HBV and HCV infection were determined by the ARCHITECT system for anti HCV and HBs Ag which is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibodies to hepatits C virus (Anti-HCV) and HBs Ag in human serum and plasma (Abbott GmbH, Wiesbaden-Delkenheim, Germany); HBV DNA was quantified by Real time PCR using artus HBV TM PCR kit (Applied Biosystems) and HCV RNA was quantified by Taq Man Assay Reagents

Table 1. Frequency of *SENV* in patients with chronic hepatitis.

Parameter	CHC (N=94)	CHB (n=18)	Controls** (n=20)
	***SENV* positive (%)**	***SENV* positive (%)**	***SENV* positive (%)**
SENV	26 /94 (27.7%)	6/18 (33.3%)	3/20 (15%)
SENV-H	10/26 (38.5%)*	2/6 (33.3%)*	0 (0%)
SENV-D	4/26 (15.4%)*	1/6 (16.7%)*	3/3 (100%)*
SENV-H/D	3/26 (11.5%)*	1/6 (16.7%)*	0 (0%)*
Negative for *SENV-H* or *D*	9/26 (34.6%)*	2/6 (33.3%)*	0 (0%)*

*The percentage was calculated against the number of *SENV* positive cases. ** P value between *SENV* positive controls and chronic hepatitis = 0.21.

(Applied Biosystems) using the 7500 fast Real Time PCR system; Polymerase chain reaction for *SENV* DNA.

Extraction of *SENV* DNA from serum

DNA was extracted from 200 µL serum using QIAamp DNA blood mini kit (Qiagen, Cat. No. 51104-Germany) according to the manufacturer's instructions

Amplification and detection

SENV DNA (349 bp) was detected by PCR according to Kojima et al. (2003). *SENV* common primers were used and were as following: forward primer AI-1F (5'-TWC YCM AAC GAC CAG CTA GAC CT-3'; W = A or T, Y = C or T, M = A or C), and reverse primer AI-1R (5'-GTT TGT GGT GAG CAG AAC GGA-3'). A 25 µl PCR mixture was used and consisted of: PCR master mix (12.5 µl), forward primer (AI – 1F) (0.5 µl), reverse primer (AI – 1R) (0.5 µl), distilled water (3 µl), extracted DNA (8.5 µl). Amplification was performed for 40 cycles, each included denaturation at 94ºC for 30 s, annealing at 52ºC for 30 s and extension at 72ºC for 60 s. This was followed by a final extension at 72ºC for 10 min to complete strand synthesis.

SENV-D DNA (231 bp) and *SENV-H* DNA (230bp) were detected by PCR with *SENV* specific primers, as previously described (Kojim et al., 2003). Type-specific primers D10S and L2AS and primers C5S and L2AS were used for *SENV-D* and *SENV-H* detections, respectively (Kojima et al., 2003). PCR mixture of 25 µl consisted of: PCR master mix (12.5 µl), forward primer (D10S) for *SENV-D* or (C5S) for *SENV-H* (0.5 µl), reverse primer (L2AS) (0.5 µl), distilled water (3 µl), extracted DNA (8.5 µl). PCR conditions for *SENV-D* and *SENV-H* genotypes were the same. Amplification was performed for 40 cycles, each included denaturation at 94ºC for 30 s, annealing for *SENV-D* at 58ºC for 30 s, for *SENV-H* at 50ºC for 30 s and extension at 72ºC for 60 s. Then, 10 min final extension at 72ºC was used to complete strand synthesis. PCR was performed in a DNA thermal cycler (HYBAID-PCR Express). The PCR products were separated using 1.5% agarose gel, stained with ethidium bromide, and visualised under a UV illuminator.

Liver histology

Liver biopsies were taken percutaneously with a 1.4 mm diameter Menghini needle and consisted of 3-5 mm long liver tissue cores. Biopsies were promptly fixed in 10% formalin, processed and embedded in paraffin blocks. Four µm sections were cut and slides were staind by hematoxylin-eosin and reticulin stains using the standard techniques. Modified hepatitis activity index (METAVIR) grading and staging were determined for each case according to the scheme given by Ishak et al. (1995).

Statistical data analysis

The statistical analysis was performed using statistical package for social sciences (SPSS) version 17.0 for Windows (SPSS, Chicago IL, U.S.A). Continuous data were expressed as means ± standard deviation (SD) and compared using Student's T test. Categorical variables were expressed as percentage and compared using chi-square ($\chi 2$) test and Fisher's exact probability test. A P value of ≤ 0.05 was considered significant.

RESULTS

Chronic HCV patients (n= 94) included 74 males and 20 females with a mean age of 47.2 ± 10.9 and chronic HBV patients (n=18) included 10 males and 8 females with a mean age of 48.4 ± 12.8. Cirrhosis was found in 50 patients with CHC (53.2%) and in 8 patients with CHB (44.4%). *SENV* DNA was detected in 28.6% of patients with chronic liver disease (32/112) and in 15% (3/20) of the control group but without a statistical significant difference (P = 0.21).

Table 1 shows the frequency of *SENV* and its genotypes H/D among the studied groups. *SENV* was detected in 27.7% of CHC cases (26/94) and in 33.3% of CHB patients (6/18). The commonest genus identified in both groups was *SENV H* in 38.5 and 33.3% respectively. However, *SENV D* was the only genus identified in controls (100%).

Combined *SENVH /D* were found in patients groups not amongst the controls. Analysis of PCR products for the presence of *SENV-H* and *SENV-D* DNA on agarose gel is shown in Figures 1 and 2.

On comparing the characteristics of patients with chronic hepatitis in relation to *SENV* viraemia (Table 2), we found that among patients with CHC without cirrhosis (n=44), *SENV* was negative in 90.9% (40/44) compared to 9.1% (4/44) *SENV* positive that was highly statistically significant (P < 0.001). On the other hand, no significant differences were found between *SENV* positive and negative CHB patients regarding any of the studied characteristics.

Regarding markers of severity of disease, we found that among patients with CHC without cirrhosis, the percentage of patients showing twofold increase in AST and ALT were significantly higher in *SENV* negative

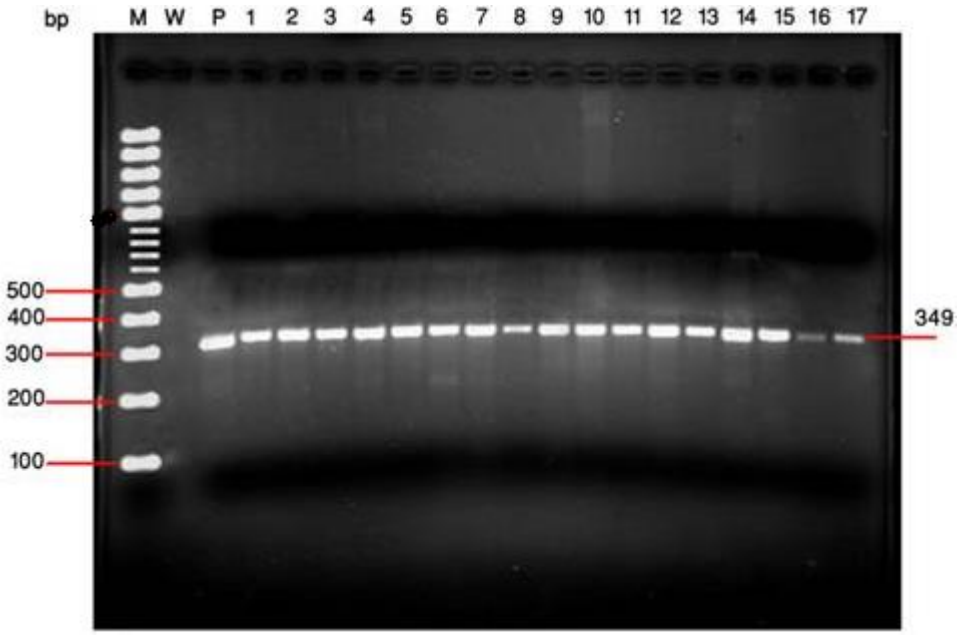

Figure 1. Detection of *SENV* DNA. Lane M, DNA 100 bp ladder; Lane W, negative control; Lanes 1- 17, positive cases in serum.

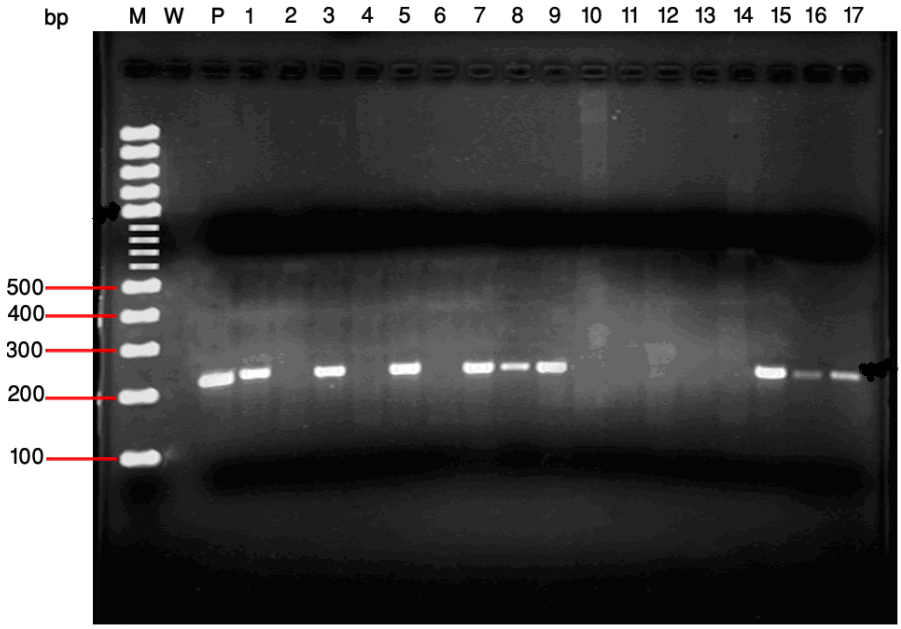

Figure 2. Detection of *SENV-D* / *H* DNA. Lane M, DNA 100 bp ladder; Lane W, negative control; Lane P, Positive control for *SENV* H; Lane 1, Positive control for *SENV* D; Lanes 2, 4, 6, 10, Negative samples for *SENV* D; Lanes 3, 5, 7-9, Positive *SENV* D; Lanes 11-14, Negative for *SENVH*; Lanes 15-17, Positive *SENV* H; Lanes 9, 17, were from the same patient.

compared to *SENV* positive patients (p=0.04 and 0.03 respectively). All the remaining clinical, laboratory and histological features were not of statistical significant difference as shown in Table 3.

Table 2. Characteristics of patients with CHC and CHB in relation to *SENV* viraemia.

Parameter	CHC patients (n = 94)				CHB patients (n = 18)			
	N	*SENV* positive (n = 26)	*SENV* negative (n = 68)	P	N	*SENV* positive (n = 6)	*SENV* negative (n = 12)	P
Age (mean±SD)	-	46.5 ± 15.9	47 ± 8.8	NS	-	51.3 ± 14.5	47 ± 12.3	NS
Sex								
Male	74	20 (27%)	54 (73%)	NS	10	4 (40%)	6 (60%)	NS
Female	20	6 (30%)	14 (70%)		8	2 (25%)	6 (75%)	
Duration of disease (years) (mean±SD)	-	3.6 ± 1.1	3.4 ± 1.5	NS	-	4.2 ± 2.2	3.4 ± 1.3	NS
History of blood transfusion (N)	8	8 (100%)	0	NS	0	0	0	-
Nature of liver disease								
Chronic hepatitis	44	4 (9.1%)	40 (90.9%)	< 0.001	10	4 (40%)	6 (60%)	NS
Liver cirrhosis	50	22 (44%)	28 (56%)	NS	8	2 (25%)	6 (75%)	NS

Table 3. Clinical, laboratory and histological features of CHC and CHB patients (without cirrhosis) in relation to *SENV* infection.

Parameter	CHC patients (N = 44)				CHB patients (N =10)			
	N	*SENV* positive (n = 4)	*SENV* negative (n = 40)	P	N	*SEN V* positive (n = 4)	*SENV* negative (n = 6)	P
Jaundice (n)	6	2 (33.3%)	4 (66.7%)	NS	2	0	2 (100%)	NS
Laboratory findings								
AST level[a]								
Mean IU/L ± SD	-	47 ± 32.6	87 ± 57.9	NS	-	76.4 ± 34.2	97 ± 78.5	NS
Elevated AST *	39	2 (5.1%)	37 (94.9%)	**0.04**	9	4 (44.4%)	5 (55.5%)	NS
ALT level[b]								
Mean IU/L ± SD	-	26.4 ± 18.9	50 ± 33	NS	-	42.8 ± 7.2	34.5 ± 12.8	NS
Elevated ALT *	32	1 (3.1%)	31 (96.9%)	**0.03**	0	0	0	-
ALP level[c]								
Mean (IU/L) ± SD	-	57.5 ± 20.2	83.2 ± 30.6	**NS**	-	74.9 ± 40	77.3 ± 33.6	NS
Elevated ALP *	4	0	4 (100%)	NS	0	0	0	NS
Albumin g/dl	-	3.2 ± 1.1	4 ± 0.7	NS	-	4.2 ± 0.4	4.3 ± 0.3	NS
Bilirubin mmol/l	-	26.8 ± 12.2	18.9 ± 10.7	NS	-	16.2 ± 5	19.7 ± 5.4	NS
Prothrombin time	-	12.6 ± 1	12.64 ± 1	NS	-	12.9 ± 1	12.5 ± 1.5	NS
Viremia (mean ± SD, log10 copies/mL)	-	5.91 ± 2.1	6.42 ± 1.2	NS	-	6.01 ± 1.46	6.68 ± 1.5	NS
METAVIR stage (n)								
1	22	0 (0%)	22 (100%)		2	2 (100%)	0	
2	14	2 (14.3%)	12 (85.7%)	NS	4	0	4 (100%)	NS
3	5	1 (20%)	4 (80%)		4	2 (50%)	2 (50%)	
4	3	1 (33.3%)	2 (66.7%)		0	0	0	
Metavier activity (n)								
A1	22	1 (4.5%)	21 (95.5%)		6	2 (33.3%)	4 (66.7%)	NS
A2	22	3 (13.6%)	19 (86.4%)	NS	4	2 (50%)	2 (50%)	

[a]Normal level: 0-32 IU/L; [b] Normal level: 0-45 IU/L; [c] Normal level: 30-120 IU/L; *Twofold or more.

Concerning cirrhotic patients, the level of ALP and serum total bilirubin were significantly higher in patients without *SENV* viraemia compared to patients with *SENV* infection (p values were 0.01 for both). Other parameters

Table 4. Comparison between cirrhotic patients with and without *SENV* infection regarding laboratory findings, severity of liver disease and liver cirrhosis related complications.

Parameter	*SENV* negative cirrhotics (N=34)	*SENV* positive cirrhotics (N=24)	p
Cause of cirrhosis			
HCV (50)	28 (56%)	22 (44%)	NS
HBV (8)	6 (75%)	2 (25%)	
Laboratory findings (mean±SD)			
AST (IU/L)	95.1 ± 61.3	118.2 ± 66.4	NS
ALT (IU/L)	52.9 ± 29.01	41.22 ± 22.5	NS
ALP (IU/L)	132.5 ± 67.6	99.4 ± 24	**0.01**
Serum albumin (g/dl)	2.2 ± 1.1	2.2 ± 0.6	NS
Serum total bilirubin (mmol/l)	90 ± 68.6	39.2 ± 20.4	**0.01**
Prothrombin time (seconds)	19.9 ± 7	17.3 ± 3	NS
Child-Pugh score	10.8 ± 2.7	10.4 ± 2.2	NS
Child-Pugh class			NS
Class A (5)	3 (60%)	2 (40%)	
Class B (7)	5 (71.4%)	2 (28.6%)	NS
Class C (46)	26 (56.5%)	20 (43.5%)	
MELD score	16.6 ± 8.9	13.7 ± 6.6	NS
Hematemesis (10)	6 (60%)	4 (40%)	NS
HE (14)	9 (64.3%)	5 (35.7%)	NS
SBP (19)	10 (52.6%)	9 (47.4%)	NS
HRS (18)	10 (55.6%)	8 (44.4%)	NS
HCC (14)	6 (42.9%)	8 (57.1%)	NS

HE, Hepatic encephalopathy; SBP, spontaneous bacterial peritonitis; HRS, hepatorenal syndrome; HCC, hepatocellular carcinoma.

regarding laboratory findings, severity of liver disease using Child-Pugh classification and liver cirrhosis related complications showed no significant differences (Table 4).

DISCUSSION

Patients with *SENV* infection develop a persistent infection that exceeds one year in approximately 45% and has been documented as long as 12 years (Umemura et al, 2002). The exact interaction of *SENV* with HCV and HBV is unclear (Tahan et al., 2003). *SENV-H* and D genotypes have been found at various rates in different populations and the role of *SEN-V* regarding the pathogenesis of liver disease is not yet known (Mu et al., 2004).

In the present study, *SENV* was detected in a considerable percentage of chronic hepatitis patients with genotype H being the most prevalent. We reported *SENV* DNA in 28.6% of patients with chronic liver disease (27.7% in CHC, 33.3% in CHB) and in 15% of the control group (3/20). The percentage of *SENV* among CHC patients is generally in accordance with the average of percentages recorded in different regions in Egypt that ranged from 13.5 to 49% (Kholeif and Fayez, 2008; Omar et al., 2008). Internationally, the *SENV* percentage was around 21% and reaching up to 69% in many previous studies (Kojima et al., 2003; Schroter et al., 2003; Yoshida et al., 2002). For the control group, we found the percentage of *SENV* to be 15% (3/20). This is in the range reported by previous Egyptian studies where it was found to be 16 and 20% among the controls (Omar et al., 2008; Sayed et al., 2006; Mohamed et al., 2011).

Regarding the CHC group, we reported *SENV* H, *SENV* D and combined H/D in 38.5, 15.4 and 11.5% respectively. This is comparable to the results of a previous Egyptian study, where the percentages were 42% (18/43), 16% (7/43) and 9% (4/43) respectively (Kholeif and Fayez, 2008). In agreement, many studies reported *SENV-H* to be more prevalent than *SENV-D* (Tezcan et al., 2009; Sayed et al., 2006; Loutfy et al., 2009). In Turkey, *SENV-H* DNA was found to be positive in 23.3% (7/30) of patients with CHC (Cakaloglu et al., 2008). On the other hand, *SENV* D was more prevalent in CHC patients (8.1%, 6/74) compared to only 5.4% (4/74) *SENV* H (Omar et al., 2008).

Concerning the CHB group, we reported *SENV* in 33.3% with *SENV* H again being the predominant genotype (33.3%) compared to only 16.7% for *SENV* D. Very limited Egyptian studies were found regarding

SENV in CHB patients. In Turkey, *SENV-H* DNA was found to be positive in 33.3% (10/30) of patients with CHB (Cakaloglu et al., 2008).

A recent study in Iran reported *SENV* in 59.3% of patients with HBV infection and in 73.5% of patients with HCV infection. *SENV-H* genotype was found to be positive in 31.39% (54/172) and 33.82% (23/68), and *SENV-D* genotype was detected in 27.91% (48/172) and 39.7% (27/68) of patients with CHB andCHC respectively (Dehkordi and Doosti, 2011).

The differences in the percentages of *SENV* detection in different countries and even in different regions in Egypt are accepted (Kholeif and Fayez, 2008; Omar et al., 2008). Similarly, in China, the prevalence varied significantly from one area to another (Tang et al., 2008). The frequency of *SENV* may vary demographically and geographically. The explanations for these differences are unknown, but they may result from interactions among behavioral, social, and biological factors (Bluthenthal et al., 1999). In addition, the difference in the rate of detection of *SENV* DNA in various studies may be due to differences in the quantity of *SENV* DNA in the sera, differences in the PCR primers used, or differences in the sensitivities of the assay systems used (Yoshida et al., 2002).

In the present study, we found that 34.6% of *SENV* (9/26) detected in CHC patients and 33.3% of *SENV* DNA (3/6) detected in CHB were not of H/D genotypes. These figures are higher than those reported in a previous study in the same hospital where *SENV* of non H/D genotype was detected in 14.3% of polytransfused patients (Mohamed et al., 2011).

Concerning the risk factors for *SENV*, we did not report any significant association with age, gender, duration of liver disease or history of blood transfusion. However, a previous study conducted on polytransfused patients in the same hospital, showed a significant difference between *SENV* positive and negative patients regarding the number of blood transfusions (Mohamed et al. 2011). This may be due to the limited number of patients giving a history of blood transfusion in this study (8/112). In addition, many 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* (Tang et al., 2008; Karimi-Rastehkenari and Bouzari, 2010). In agreement with our results, another Egyptian study reported a statistically insignificant difference between *SENV* positive and *SENV* negative liver patients regarding age and sex (Kholeif and Fayez, 2008). In Turkey, Cakaloglu et al also found no significant difference in the clinical features between *SENV-H*-positive and -negative patients with chronic viral hepatitis (Cakaloglu et al., 2008).

In the current study, we reported many important findings suggesting the absence of deleterious effects of *SENV* on the progression of chronic liver disease or even a possible protective role of *SENV* infection in patients with CHC. We demonstrated that *SENV* was negative in 90.9% of patients with CHC compared to 9.1% *SENV* positive among such group giving a statistically significant difference. In addition, we found a significantly higher percentage of patients showing twofold or more increase in the AST and ALT among *SENV* negative CHC patients (without cirrhosis) compared to *SENV* positive patients (P=0.04 and 0.03, respectively). Even for cirrhotic patients, the levels of ALP and serum total bilirubin were significantly higher in *SENV* negative patients compared to *SENV* positive patients (P values=0.01). A similar conclusion was reported in a previous Egyptian study where the prevalence of *SENV* infection among patients who have recovered from HCV infection was 61% (11/18) compared to 50% in HCV viremic patients (Loutfy et al., 2009). This finding was also observed in another study that reported that HCV was less prevalent among patients with *SENV-H* viremia (14%) than among patients without *SENV-H* viremia (34%) in an area of high HCV endemicity (Umemura et al., 2001). They also suggested a possible protective role of *SENV* against HCV or assistance with HCV clearance by some sort of virus to virus interaction, making *SENV* worthy of further studies on larger scales. For our patients with CHB, the effect of *SENV* was not conclusive; this may be due to the small number of patients included in the study.

Apart from the previously mentioned parameters, there were no statistically significant differences between *SENV*-positive and *SENV* negative chronic hepatitis patients regarding the demographic data and other laboratory findings. This is in agreement with many studies. A previous Egyptian study reported a statistically insignificant difference between CHC patients and HCV related HCC patients regarding *SENV* viraemia. They also reported no statistically significant difference regarding ALT, serum bilirubin, serum albumin and prothrombin time (Kholeif and Fayez, 2008). The same finding regarding the biochemical parameters were also reported by Alam El-Din et al. (2007). In addition, Yoshida et al. (2002) reported no significant differences between *SENV*-positive and *SENV* negative patients regarding serum albumin, total bilirubin and transaminase levels. Also, many studies reported absence of significant difference in the blood biochemical parameters between the *SENV* DNA-positive and -negative chronic hepatitis patients (Tangkijvanich et al., 2003; Moriyama et al., 2005).

Among our patients, we reported no histopathological differences and no liver cirrhosis related complications including HCC between *SENV* positive and negative chronic hepatitis patients. In concordance, Tangkijvanich et al. (2003) also reported no differences between *SENV*-infected and non-infected patients regarding severity of chronic liver disease and HCC. Recently, no evidence has been produced to indicate that SEN virus causes HCC (Kew, 2013). On the contrary, another study documented that *SENV* co-infection may influence the histopathological features of the livers of patients with

CHC but does not affect the outcome of patients with type C chronic liver disease. The histological features of the livers of *SENV* DNA-positive patients included more severe parenchymal inflammatory cell infiltration and more immune response (Moriyama et al., 2005).

The exact role of this virus in the pathogenesis of chronic liver diseases is not yet confirmed. Several studies reported that *SENV* does not seem to contribute to the pathogenesis of liver disease or worsens the course of coexistent liver disease or lead to the development of HCC from chronic liver disease (Yoshida et al., 2002; Akiba et al., 2005). Others suggested a protective role of *SENV* against HCV (Umemura et al., 2001; Loutfy et al., 2009). On the other hand, a recent Indian study reported that *SENV* appeared to cause liver damage in patients with hepatitis, but the number of hepatitis patients coinfected with *SENV* were very limited in that study (5 HBV and 3HCV) (Rizvi et al., 2013). So, further studies are needed to ascertain the association of *SENV* with liver disease.

From the present study we conclude that *SENV* virus does not worsen the progression of chronic viral hepatitis. A possible protective effect of *SENV* in CHC patients was reflected by changes in liver enzymes without histo-pathological changes which needs to be emphasized by further studies on larger scales

Conflict of interests

The authors did not declare any conflict of interest.

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