Prevalence of SEN Virus Infection in Multitransfused Patients in Assiut University Hospitals, Egypt

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Abstract: SENV is a blood- borne, circular ss DNA virus and possessing nine genotypes (A to I). Among nine genotypes, SENV-D and SENV-H genotypes have the strong link with patients with non (A-E) hepatitis infections. Recently, the identification of SEN virus (SENV) as a possible etiologic agent of parenteral transmission hepatitis let to the study of the prevalence of such agent. This study compared SENV prevalence and its two important genotypes (D&H) which might be pathogenic in high risk subjects including blood transfused patients and hemodialysed patients and low risk subjects as healthy blood donors.

Subjects and methods: This study included 75 multitransfused patients, 60 of them were hemodialysed and the remaining were blood diseased including haemophilics, anaemics and leukemics. The study included also 25 healthy blood donors as a control. They were enrolled consecutively at the department of Internal Medicine, Assiut University Hospital. The sera were separated and SENV DNA was detected by polymerase chain reaction.

Results: A higher prevalence of SENV infection was detected *in* patients groups than in blood donors (46.7% versus 20%).No significant relation was found between SENV infection and age, duration of haemodialysis or liver enzymes. However, there was significant difference between SENV positive and negative patients as regards gender and number of blood transfusions.

Conclusions: SENV is commonly present in blood transfused and haemodialysed patients attended to Assiut University Hospitals as well as in blood donors at comparable rates. SENV infection has been found in only 20% of blood donors but in 46.7% of patients. The results also indicated that other possible routes of SENV infection other than blood transfusion may be included. Its pathogenic role in causing hepatitis is not documented, so far it can be considered as simple guest till further studies have been done.

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1. Introduction:

Hepatitis viruses research started more than fifty years ago (Arie, 1996). As candidates for unknown hepatitis viruses, 2 novel isolates were identified from patients with non (A-E) hepatitis and were designated hepatitis G virus (HGV) and TT virus (TTV) (Nishizawa et al., 1997). When these viruses were discovered, they were expected to account for most of the residual cases of acute or chronic hepatitis that were unrelated to hepatitis (A–E) viruses. Although both HGV and TTV spread universally, there has been no confirmed demonstration of an etiologic association between these viruses and human diseases (Simmonds *et al*., 1998). SEN virus (SENV) is the latest viral agent that has been proposed as a cause of non (A-E) hepatitis. SEN virus is a blood-borne virus that was discovered in 1999 by investigators at DiaSorin Biomolecular Research Institute, Saluggia, Italy, in their search for a viral cause of those cases of post-transfusion hepatitis that are not due to the hepatitis B virus (HBV) or hepatitis C virus (HCV) (Alunan , 1999).

The discovery of SENV by an Italian research group, led by Dr. Daniele Primi, was announced at a press conference in July 1999 without the support of any published empirical data (Allain, 2000). The nomenclature of the virus is derived from the initials of the infected patient, a human immunodeficiency virus–infected injection drug user from whom the virus was first isolated. The first publication of an European patent of the nucleic acid sequence of SENV was on 18 May, 2000 (Primi et al., 2000). Reports of subsequent studies by other investigators were in 2001 (Tanaka *et al*., 2001).

The virus is subgrouped into eight genotypes, SENV- A to H. The ninth genotype (SENV-I) has been identified (Fiordalisi *et al*., 2000). The routes of SENV infection might be mostly parenteral, e.g. transmission by blood transfusion, intravenous drug use or haemodialysis (Umemura *et*

al., 2001a). Transplantation of organs or hematological progenitor cells can also represent a potential risk of infection transmission. SENV has the same transmission modes as HBV and HCV (Yoshida *et al*., 2001).Transfusion significantly increases the relevance of SENV infection. Many studies in different countries revealed that SENV infection is high in patients on maintenance haemodialysis. It is possible; however, that SENV may be transmitted via other means (Hsu *et al*., 2007).

The aim of this study was to determine the prevalence of SEN virus infection among multitransfused patients compared to that of healthy blood donors volunteers in Assiut University Hospitals using polymerase chain reaction (PCR), to determine the genotype of SEN virus detected strains whether SENV-D or SENV-H and also to determine the effect of SEN virus on liver enzymes in multitransfused patients to detect its possible role in causing hepatitis.

2. Subjects and Methods

This study included 75 multitransfused patients, 60 of them were haemodialysed and the remaining 15 were blood diseased including haemophilia, anemia and leukaemia. The study included also 25 healthy blood donors as a control. They were enrolled consecutively at Department of Internal Medicine, Assiut University Hospital and were subjected to clinical examination.

The seventy five patients included in this study were classified into 2 groups, while the third group was the control group: Group I: included 15 blood diseased patients with haemophilia, anemia and leukemia. Group II: included 60 haemodialysed patients. Group III: represented the control group consisting of 25 healthy blood donors. Exclusion criteria: all enrolled subjects were negative for known serologic markers of hepatitis B and C, including IgM antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg) and antibodies to HCV (anti-HCV).

Specimen collection and processing:

Blood specimens (3-5 ml volume) were collected in a clean test tube without any anti-coagulant. Each blood specimen was spun down, within 1 hour of its collection, at 3000 r.p.m. for 10 minutes. Each separated serum was collected and stored at -20ºC.

Extraction of viral DNA:

Viral DNA was extracted from 200 µl serum with QIAamp DNA blood mini kit (Qiagen, Cat. No. 51104- Germany).

Detection of SENV DNA:

SENV DNA was detected by polymerase chain reaction (PCR) with SENV specific primers, as described by Umemura *et al*. (2001a) and Kojima *et al*. (2003). Specific primers **:**(Metabion International AG, D-82152 Martinsried/Deutschland). Sense primer: AI-1F (5'-TWCYCMAACGACCAGCTAGACCT-3'), and antisense primer: AI-1R (5'-GTTTGTGGTGAGCAGAACGGA-3')NB: W= (A or T**),** Y=(C or T), M= (A or C) were used. PCR mixture of 25 µl 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 sec, annealing at 52ºC for 30 sec and extension at 72ºC for 60 sec. Then 10 min final extension at 72ºC was used to complete strand synthesis.

Genotyping of SENV using PCR:

SENV-D DNA and SENV-H DNA were detected by PCR with SENV specific primers, as described by Kojima *et al*., (2003). Specific primers **:**(Metabion International AG, D-82152 Martinsried/Deutschland). For SENV-D detection, the primers D10S and L2AS were used. Sense primer: D10S (5'-GTAACTTTGCGGTCAACTGCC-3') and antisense primer: L2AS (5'-CCTCGGTTKSAAAKGTYTGATAGT-3') For SENV-H detection, the primers C5S and L2AS were used. Sense primer: C5S (5'-GGTGCCCCTWGTYAGTTGGCGGTT-3') and antisense primer: L2AS (5'-CCTCGGTTKSAAAKGTYTGATAGT-3')

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**).**Amplification was performed for 40 cycles, each included denaturation at 94ºC for 30 sec, annealing for SENV-D at 58ºC for 30 sec, for SENV-H at 50ºC for 30 sec and extension at 72ºC for 60 sec. Then 10 min final extension at 72ºC was used to complete strand synthesis. PCR was performed in a DNA thermal cycler **(**Omnigene TR3, CM220, United Kingdom). For visualization, all of the PCR products generated from PCR amplification were electrophoresed and sized on1.5% agarose gel using 100 bp DNA ladder as DNA molecular weight marker.

Liver function tests:

For measuring alanine aminotransferase (ALT): (BioMed Diagnostics) (Henry, 1964), aspartate aminotransferase (AST): (BioMed Diagnostics) (Tietz, 1976) and total bilirubin (Diamond Diagnostics 30175 Hannover, Germany) (Kaplan *et al*., 1984).

Statistical Analysis:

The data were entered and analyzed using the Statistical Package for Social Science (SPSS) version 16 for windows. For qualitative variables, frequencies and percentages were used.X^2 -test (chi-square test) was used to compare the proportions between the groups. For quantitative variables, mean (x') and standard deviation (SD) were used. Independent T-test, ANOVA and fisher exact test were used to compare between the groups. Significance was discriminated by P value < 0.05.

3. Results

The 75 patients (50 males and 25 females with mean age 45± 13.6) included in this study were classified into 2 groups; one group included 15 blood diseased patients (BD) with leukemia, hemophilia and anemia (3 males and 12 females with mean age 45.8± 18.2). The other group included 60 haemodialysed patients (HD) (47 males and 13 females with mean age 45.4± 12.8). The study was conducted also on 25 healthy blood donors (20 males and 5 females with mean age 31.3± 10.3) as a control group.

SENV was detected in 46.7%of the patients (35 of 75 patients) while it was detected in 20% of the blood donors (5 of 25) with a statistically significant difference as shown in table (1). SENV was detected in 45%of haemodialysed patients (27 of 60 patients) while it was detected in 53.3% of blood diseased patients (8 of 15) as shown in table (2). SENV DNA positive samples showed one band at 349 bp DNA fragment when examined by gel electrophoresis (Fig. 1A).

SENV-D was detected in 14.3%of SENV positive patients (5 of 35 patients ; 3 haemodialysed patients and 2 blood transfused patients) while it was detected in all of SENV positive blood donors (100%) as shown in tables (3&4). SENV-D DNA positive samples showed one band at 231 bp DNA fragment when examined by gel electrophoresis (Fig. 1B).

SENV-H was detected in 14.3%of SENV positive patients (5 of 35 patients; 3 haemodialysed patients and 2 blood transfused patients). Whereas none was detected in the control group as shown in tables (3&4). SENV-H DNA positive samples showed one band at 230 bp DNA fragment when examined by gel electrophoresis (Fig. 1C).

SENV-D/H co-infection was detected in 57.1%of SENV positive patients (20 of 35 patients; 19 haemodialysed patients and 1 blood transfused patient), whereas none was detected in the control group as shown in tables (3&4).

There is no significant difference in mean age between SENV positive and negative patients while it is evident significant difference between SENV positive patients and SENV negative patients, as regards gender (Table 5).

Concerning the risk factors for patients, it was found highly significant statistical difference regarding the mean number of blood transfusions which was 2.33± 0.6 vs. 11± 7.6 in SENV positive and negative patients respectively (Table 5). The mean number of blood transfusion was nearly similar among SENV – D positive patients, SENV –H and SENV –D/H patients (2.9, 2.5 and 2), respectively, as shown in table (6).

The mean duration of haemodialysis was 8.1± 4.9 in SENV positive patients vs. 7.2± 2.7 in SENV negative patients with no significant statistical difference between them (Table 5). Higher mean concerning duration of haemodialysis was noticed among SENV–D positive patients than among SENV- H or SENV- D/H patients (10± 2.1 vs. 6± 1.4 and 8.1± 4.9) respectively as shown in table (6).

The biochemical parameters as regards liver enzymes (ALT, AST and total bilirubin) didn't significantly differ between SENV positive and negative patients as shown in table (5). Table (6) showed no statistically significant difference between SENV – D positive, SENV – H positive and SENV – D/H positive patient as regards AST, total bilurbin and mean duration of haemodialysis. However, mean AST of SENV – H infected patients was slightly higher than other types of infection .However, statistically significant difference occurred among SENV- D positive , SENV –H positive and SENV – D/H positive patient as regards ALT in spite of their normal values.

Concerning the two patients groups and the liver enzymes values, there is no statistically significant difference either between SENV positive and negative haemodialysed patients or blood transfused patients (Table 7). Table (8) showed no statistically significant difference between SENV positive and negative blood donors.

Table (1): Prevalence of SENV infection using PCR in patients and control groups

SENV infection	Patients (n=75)	Control (n=25)	*P*-value*
Positive	35(46.7%)	5(20%)	0.02
Negative	40(53.3%)	20(80%)	

Fisher exact test*

Table (2): Prevalence of SENV infection in different patients groups and the control group

Group	No. of subjects	SENV positive	SENV negative	*P*-value*
Haemodialysed patients	60	27(45%)	33(55%)	0.56
Blood diseased patients	15	8(53.3%)	7(46.7%)	0.63
Control group	25	5(20%)	20(80%)	0.0001

Fisher exact test*

Table (3): Prevalence of SENV genotypes in SENV positive subjects groups.

	SEN V positive		*P*-value*
	Cases (n=35)	Control (n=5)	
SENV-D	5(14.3%)	5(100%)	0.0006
SENV-H	5(14.3%)	0(0%)	
SENV-HD	20(57.1%)	0(0%)	
Negative for SENV- D or H	5(14.3%)	0(0%)	0.35

Fisher exact test*

Table (4): Prevalence of SENV genotypes in patients and control groups.

Group	SENV-D	SENV-H	SENV-D/H	Negative for SENV- D/H
Haemodialysis patients	3 (11.1%)	3 (11.1%)	19 (70.4%)	2 (7.4%)
Blood diseased patients	2 (25%)	2 (25%)	1 (12.5%)	3 (37.5%)
Control group	5 (100%)	0 (0%)	0 (0%)	0 (0%)

Table (5): Relationship between gender,age, risk factors, liver enzymes and SENV infection

The variable	SENV Positive (N=35)	SENV Negative (N=40)	*P*-value
Gender • **Male** • **Female**	 18 (51.4%) 17 (48.6%)	 32 (80%) 8 (20%)	0.003*
Age	50.6 ± 10.1	44.1 ± 9.6	0.09*
Risk Factors • **Duration of haemodialysis (years)** • **No of blood transfusions (units)**	 8.1 ± 4.9 2.33 ± 0.6	 7.2 ± 2.7 11 ± 7.6	 0.4 0.0001*
Liver function tests • **ALT (U/L)** • **AST (U/L)** • **Total Bilirubin (mg/dl)**	 7.4 ± 5.2 11.9 ± 4.1 0.26 ± 0.03	 8.7 ± 4.9 9.1 ± 4.5 0.22 ± 0.02	 0.5 0.1 0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase.
*Fisher exact test

Table(6):Relationship between SENV genotypes infection and different variables in the patient groups.

The variable	D (n=5)	H (n=5)	H\D (n=20)	P-value
Risk Factors • **Duration of haemodialysis** • **Amount of blood transfusion**	10 ± 2.1 2.9	6 ± 1.4 2.5	8.1 ± 4.9 2	0.08*
Liver Function Tests • **ALT (U/L)** • **AST (U/L)** • **Total Bilirubin (mg/dl)**	4 ± 1.3 10 ±4.2 0.02 ± 0.008	10.5 ± 9.2 17.5 ± 2.1 0.02 ± 0.0001	7.1± 5.02 11.5 ± 3.8 0.3 ± 0.03	0.04** 0.1** 0.7**

AST, aspartyl transaminase, ALT, alanine amino-transaminase. *Fisher exact test, ** ANOVA

Table (7): Multitransfusion and haemodialysis impacts on the prevalence of SENV infection and the liver function tests in patients groups.

	Liver Function test	SENV Positive	SENV negative	p-value
Haemodialysed patients	ALT (U/L) AST (U/L) Total Bilurbin (mg/dl)	7.5 ± 5.4 12.2 ± 4.3 0.2 ± 0.03	9.8 ± 5.4 9.6 ± 4.9 0.2 ± 0.1	0.5 0.06 0.8
Multi-transfused patients	ALT (U/L) AST (U/L) Total Bilirubin (mg/dl)	7.3 ± 5.8 11 ± 3.5 0.5 ± 0.3	5.7 ± 3.3 7.9 ± 3.2 0.3 ± 0.08	0.6 0.4 0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase

Table (8): Comparison of SENV positive and negative blood donors (control subjects) as regards liver enzymes.

Liver Function tests	SENV positive (No.=5)	SENV negative (No.=20)	p-value*
ALT (U/L)	5.5 ± 2.1	5.4 ± 3.1	0.9
AST (U/L)	9 ± 3.4	9.9 ± 2.1	0.9
Total Bilirubin (mg/dl)	0.4 ± 0.3	0.3 ± 0.08	0.7

AST, aspartyl transaminase, ALT, alanine amino-transaminase

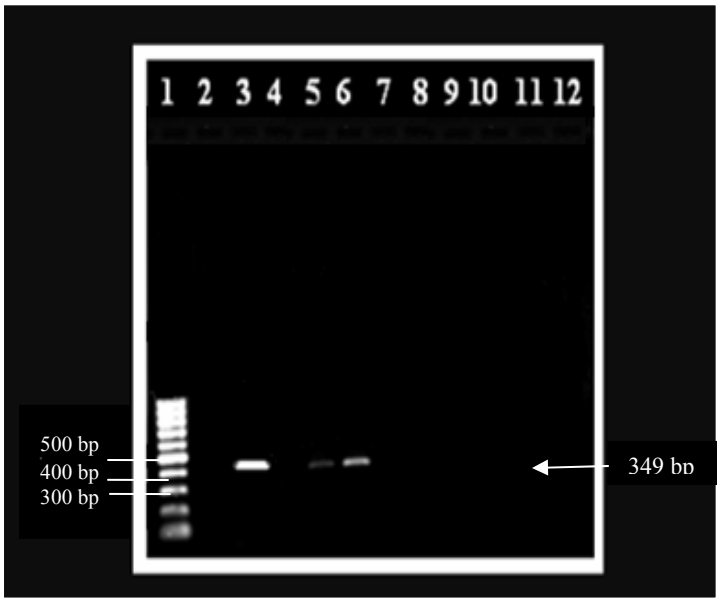

Figure (1A): **Detection of SENV DNA**
Lane (1): DNA 100 bp ladder.
Lane (2): Negative control.
Lane (3): Positive control.
Lanes (5) and (6): Positive cases.
Lanes (4), (7)-(12): Negative cases.

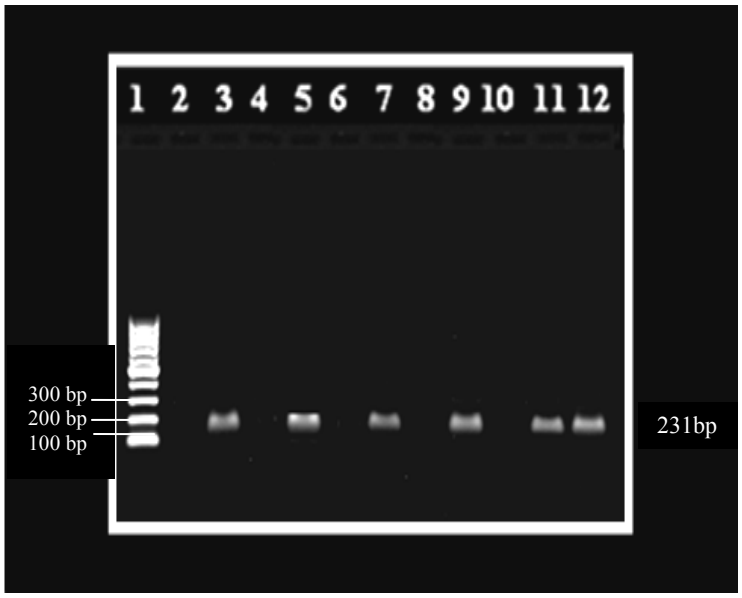

Figure (1B): **Detection of SENV-D DNA**
Lane (1): DNA 100 bp ladder.
Lane (2): Negative control.
Lane (3): Positive control
Lanes (4), (6), (8) and (10): Negative cases.
Lanes (5), (7), (9), (11) and (12): Positive cases

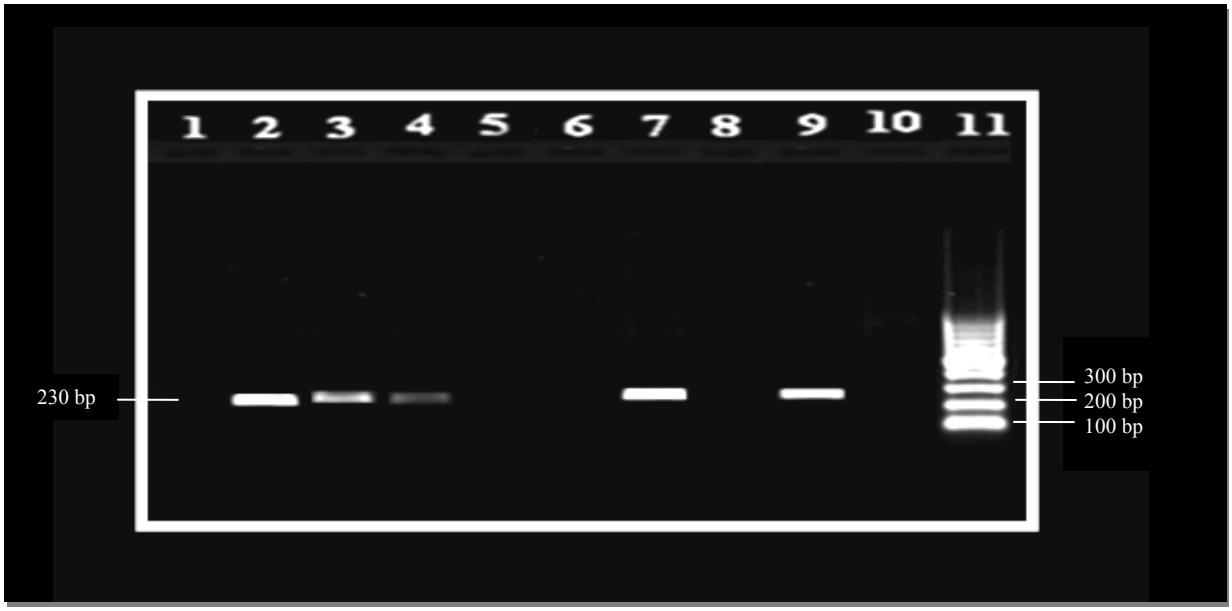

Figure (1C): Detection of SENV-H DNA
Lane (1): Negative control.
Lane (3), (4), (7) and (9): Positive cases.
Lane (11): DNA 100 bp ladder.
.Lane (2): Positive control.
Lanes (5), (6), (8) and (10): Negative cases.

4. Discussion:

SENV is a blood- borne ,circular ss DNA virus and possessing nine genotypes (A to I). Among nine genotypes, SENV-D and SENV-H genotypes have the strong link with patients with non (A-E) hepatitis infections (Karimi-Rastehkenari and Bouzari, 2010).

The current study compared the prevalence of SENV in blood transfused, haemodialysed patients and blood donors. The study was conducted on 75 patients (50 males and 25 females) and 25 healthy blood donors (20 males and 5 females). SENV was detected in 46.7% of patients compared to 20% of the blood donors. Many investigators in different countries mentioned data in agreement with the present study as infection rates in the blood donors were 10-20% in Japan (Shibata et al .,2001), 14-20% in Taiwan (Kao *et al*., 2002), 13% in Italy (Pirovano et al., 2002), 24% in Greece (Umemura *et al*., 2003), 31% in China (Mu *et al*., 2004), 16% in Egypt (Sayed et al., 2006), 25% in Turkey (Serin *et al*.,2006) and (Sharifi *et al*.,2008).

However, this rate was higher than that of blood donors in other reports from different parts of the world; in Italy (2%) (Mushahwar, 2000), in the United States (1.8%) (Umemura *et al*., 2001a), in Germany (8%) (Schröter *et al*., 2002) and (10%) in Egypt (Loutfy *et al*., 2009). The reasons for these demographic differences are unclear, but they make it essential to compare case and control subjects from areas of similar endemicity (Umemura *et al*., 2003).

Patients on maintenance haemodialysis (HD) should be at increased risk of such an infection, as they are for HDV and HBV infections (Wreghitt, 1999) since the virus may be transmitted parenterally (Umemura *et al.,* 2001a). In support of this view, the overall detection of SENV DNA in HD patients of this study was higher than that observed in healthy blood donors (45% vs. 20%).This is in agreement with Kobayashi *et al*., (2003) who reported a prevalence of 37.6% among HD patients in Japan, Dai *et al*., (2005) reported 61.6% in Southern Taiwan, Pirovano et al., (2005) reported 40.9% in Italy and Loutfy *et al*., (2009) reported 52.4% in Egypt. In reverse, other investigators reported lower prevalence as Schröter *et al*., (2003) who reported prevalence of 10.9% among HD patients and Hsu *et al*., (2007) reported prevalence of 27.7% among peritoneal dialysis (PD) patients.

In the present study, the prevalence of SENV among blood transfused patients (patients with hemophilia, anemia and leukemia) was higher than that of blood donors (53.3% vs.20%). This is in agreement with Kao *et al*., (2002) who reported that the prevalence of SENV among blood transfused patients was high being 68% in hemophilic patients and 90% in thalassemic patients. These patients were frequently exposed to blood and blood products, confirming the importance of the parenteral route for SENV transmission. This is in agreement with Karimi-Rastehkenari and Bouzari, (2010) who reported that SENV viremia was significantly higher among thalassemic patients than healthy individuals.

On the other hand, Umemura *et al*., (2003) reported lower prevalence (35%) in patients with hepatitis associated aplastic anemia (HAA).

The present study revealed that SENV-D was the only genotype detected in all SENV positive subjects of the control group (100%) while SENV-H was not detected in this group. Therefore, combined infection was not detected in this group. This is in agreement with a study done in US by Umemura *et al*. (2001b) and also in Japan and Greece (Umemura et al., 2003). In contrast to the present investigation, SENV-H was the predominant strain in blood donors in the US (Umemura *et al*., 2001b) and Taiwan (Kao *et al.,* 2002). Schröter *et al*. (2002) reported that the prevalence of SENV-H was 16.8% among blood donors. Thom et al. (2010) detected SENV –H prevalence in 45.4% of Ghanaian blood donors.

Different rates of the two genotypes (D&H) were mentioned by other investigators. The prevalence of SENV-D mentioned by Kobayashi *et al*, (2003) was 77%, that of SENV-H was 15% and that of both SENV-D/H was 8% in the control group. In Turkey, Serin *et al*., (2005) detected SENV-D in 2 (40%) of 5 SENV positive subjects and SENV-H was detected in 3 (60%) of 5 SENV positive subjects in the control group.In another study, Serin *et al*., (2006) reported that the prevalence of SENV-D was 10% while SENV-H was 15% in the blood donors.

In a study done in Egypt by Sayed *et al.,* (2006), SENV-D was detected in 4 % of blood donors while SENV-H was detected in 12%of blood donors. Co-infection with both variants was not detected in the blood donors, this supported the present study. Borawski *et al*. (2006) detected SENV-H in 2 % of control subjects in Poland. In Iran, Sharifi *et al*. (2008) detected SENV-D/H in 23% of blood donors, while Karimi-Rastehkenari and Bouzari (2010) reported that frequency of SENV-H viremia was significantly higher than SENV-D among healthy individuals.

All these variations may be referred to that high prevalence of SENV infection can be attributed to only some of the SENV strains; for example, SENV-B, SENV-A and SENV-E which are found among healthy blood donors and do not appear to be related to non (A-E) hepatitis (Allain *et al*., 2002).This discrepancy is postulated to be due to geographical distribution of SENV variants and differences between regions in the same country (Dai *et al*., 2005).

Either SENV-D or SENV-H was detected in SENV positive patients in a similar percentage (14.3%). However, combined infection with both variants was higher than either genotype monoinfection (57.1% vs. 14.3%). The results of this study were in agreement with a study done by Quiros Roldan *et al*., (2005) who found SENV-D and SENV-H in a similar percentage (16%). In contrast, , many investigators as Kao *et al*., (2002) reported that the prevalence of SENV-H was 2-7 times higher than that of SENV-D in different subjects, and mixed SENV-D/H infection was not common. Schréter *et al*., (2006) showed 34.7% SENV-H positive cases.

A study on the prevalence of SENV among patients undergoing haemodialysis (HD) in Poland revealed that SENV-H viraemia was prevalent in 40% of HD patients (Borawski *et al*., 2006). The present study revealed that either SENV-D or SENV-H was detected in SENV positive patients on HD in a similar percentage (11.1%). The proportion of SENV-H positive HD patients is similar to that found in HD patients from Germany (12.8%) (Schröter *et al*., 2003), but lower than that found in Japanese HD patients (38%) (Kobayashi *et al*., 2003).

These results strongly suggest that infection with one SENV variant most likely does not protect against infection with another SENV variant (Pirovano *et al*., 2005). In Egypt, as previously reported by Loutfy *et al*., (2009), 61% of HD patients were positive to SENV-H only,4% were positive to SENV-D only, and 36% were positive for both SENV-H and SENV-D. Since patients who have been on HD for the longest period of time are likely to have received more blood transfusion, one could expect that the presence of SENV DNA correlates with the HD treatment.

It was demonstrated from this work that there was no relationship between SENV positivity and the length of time on HD. This indicated that the blood transfusion may not be the only important route of SENV transmission in these patients but also other routes may be included. Evidence to support transmission of SENV by blood transfusion has been reported (Shibata et al., 2001).

In the present investigation, the number of blood transfusions in SENV positive patients was lower than that of SENV negative patients denoting that the number of blood transfusions is probably not a risk factor. Similar result is obtained by Loutfy *et al*., (2009) who revealed no association between SENV infection and duration of hemodialysis. However, Schréter *et al*. (2006) found that the number of blood transfusions was significant risk factor.

The present work revealed that there was no association between age and prevalence of SENV infection as there was no significant difference between SENV positive patients and SENV negative patients as regards age. However, some authors described an age – specific prevalence of SENV in adults (Kao et al., 2002).

SENV infection was found in nearly similar proportions among males and females. In spite of this, significant difference was noticed between SENV positive patients and SENV negative patients as regards gender. This was in agreement with data of many investigators, Yoshida *et al*. (2002) mentioned no significant differences in age and gender between SENV positive and SENV negative patients with non B and non C chronic liver disease. Results of many other studies support the previous data (Mikuni *et al*., 2002; Pirovano *et al*., 2002; Kobayashi *et al*., 2003; Quiros Roldan *et al*., 2005; Serin et al., 2005 and Borawski et al., 2006). In Egypt, Loutfy et al., (2009) revealed no association between SENV infection and age or sex of HD patients. In contrast, Kobayashi *et al*., (2003), Chiou *et al*., (2006), Schréter *et al*., (2006) and Spataro *et al.* (2006) described a notable difference in SENV prevalence according to gender with a higher proportion of males among SENV positive patients.

On comparison the laboratory parameters of liver injury in SENV positive and negative patients, it was not observed any differences in liver *al* enzymes values (ALT, AST and TB). Similar to other studies (Shibata *et al*., 2001; Umemura *et al*., 2001b; Yoshida *et al*., 2002; Kao et al., 2002; Kobayashi *et al*., 2003; Mu *et*., 2004; Sagir *et al.,* 2004; Borawski *et al*., 2006 and Schréter *et al*., 2006), it has not been observed any influence of SENV infection on the worsening of laboratory findings in the patients group, hence no confirmation of a pathogenic role of SENV in liver injury.

Chiou et al. (2006) reported also that SENV viraemia was not associated with elevated liver enzymes in thalassemia patients. High levels of ALT, AST and TB in SENV positive patients compared with SENV negative patients have been reported. However, these findings were not statistically significant (Pirovano *et al*., 2002 and Serin *et al*., 2005). So they concluded that SENV did not seem to contribute to the pathogenesis of liver diseases.

5. Conclusions:

SENV is commonly present in blood transfused and haemodialysed patients attended to Assiut University Hospitals as well as in blood donors at comparable rates. SENV infection has been found in only 20% of blood donors but in 46.7% of patients. The results also indicated that other possible routes of SENV infection other than blood transfusion may be included. Its pathogenic role in causing hepatitis is not documented, so far it can be considered as simple guest till further studies have been done.

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