Detection of SEN virus (SEN-V) and Torque Teno virus (TTV) Co-Infection and Liver Enzyme in a Sample of Hepatitis Patients.

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Abstract:

In addition to well established hepatitis viruses in reviews (A, B, C, D, E), there is increase evidences suggested the existence of new hepatitis viruses that play a role in this disease. This study aimed to determine the synergistic effect of concordant infection with SEN virus and Torque Teno virus in patients with hepatitis and apparently healthy blood donors as control. Sera were collected from 50 patients who had hepatitis type B or C as a case group and from 50 apparently healthy blood donors as control. All samples were tested by polymerase chain reaction (PCR) for detection of SEN-V DNA, while TTV antigen were tested by immunoassay. In addition, ALT and AST enzymes have been tested using biochemical test. TTV and SEN-V co-infections had been detected in hepatitis patient and apparently healthy blood donors with exposure rate (26%) and (4.65%), respectively. Current study indicated that there were no significant differences between the mean level of ALT or AST enzymes and the SENV/TTV co-infection. Also, there was no significant associated between SENV/TTV co-infection and risk factors under study.

Key words: Liver disease, SEN-V, TTV, Hepatitis B virus, Hepatitis C virus.
List of abbreviation: SEN-V = SEN virus, Torque Teno virus = TTV AST= Aspartate Aminotransferase, AST = Aspartate Aminotransferase.

الكشف عن العدوى المشتركة بفيروس SEN و فيروس TT و انزيمات الكبد في عينة من المرضى المصابين بالتهاب الكبد الفيروسي

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الخلاصة:

بالاضافة الى فيروسات التهاب الكبد المعروفة سابقا (A، B، C، D، E)، هناك العديد من الأدلة تشير الى وجود فيروسات جديدة لها دور في هذا المرض. تهدف هذه الدراسة إلى تحديد التأثير التآزري للعدوى المشتركة لفيروسي SEN-V/TTV في مرضى التهاب الكبد الفيروسي والمتبرعين بالدم الاصحاء. تم جمع عينات مصل الدم من 50 مريض بالتهاب الكبد نوع B أو C كحالات ومن 50 من المتبرعين بالدم الاصحاء كمجوعة سيطرة. تم اختبار جميع العينات عن طريق تفاعل سلسلة البلمرة المتسلسل (PCR) للكشف عن الحمض النووي لفيروس SEN-V، بينما تم الكشف عن مستضد TTV بواسطة المقايسة المناعية. بالإضافة إلى ذلك، تم التحري عن انزيمات الكبد بواسطة الفحوصات البايوكيميائية. تم الكشف عن العدوى المشتركة بين TTV و SEN-V في مرضى التهاب الكبد والمتبرعين بالدم الاصحاء في (26٪) و (4.65٪)، على التوالي. وجدت الدراسة الحالية أنه لا توجد علاقة ذات دلالة إحصائية بين متوسط ALT أو AST و إيجابية SENV/TTV . تشير النتائج ايضا إلى أن وجود SENV/TTV لم يرتبط بعوامل الخطر قيد الدراسة .

الكلمات المفتاحية: أمراض الكبد ، فيروس SEN-V ، فيروس TTV ، فيروس التهاب الكبد نوع B ، فيروس التهاب الكبد نوع C.

Introduction:

SEN virus (SEN-V) is a relatively recently discovered virus that thought to be essentially connected with hepatitis (1). SEN-V has been described as a blood borne infection that has a worldwide incidence (2). SEN-V was detected in 67%, 41% among patients with Hepatitis C virus (HCV) and Hepatitis B virus (HBV), respectively as well as in 16% among healthy blood donors (3,4).

About 20% of hepatic infections are not associated with hepatitis viruses (A–E) and might be due to other viruses. The genome of SEN-V is similar to that of the Torque Teno virus (TTV) (5). Both of them are circular single stranded DNA viruses that belong to *Anelloviridae* family (6,7). Wide ranges of SEN-V and TTV infections was found in patients with chronic liver disease, thalassemia, acquired immunodeficiency syndrome (AIDS), also in intravenous drug users and hemodialysis patients(8).

There is expanding confirmations recommended a potential role of TTV infection in liver disease. A study done by Nishizawa *et al*. found that the presence of TTV-DNA in the sera of patients with non (A-E) hepatitis reveal a close correlation with alanine aminotransferase (ALT) level (9). In addition, Okamoto *et al.* reported that TTV-DNA levels in liver tissue were equal or hundred times more than those detect in serum which indicated that TTV replicated in the liver (10). The frequency of TTV-DNA in hepatitis B and hepatitis C infected patients is 90.75% and 84.9%, respectively, as well as 81.4% in apparently healthy individuals (11).

SEN-V and TTV were thought to be linked to hepatitis, even this significant association does not establish causality, it is probable on the basis of many studies that these viruses are not the cause of non (A-E) hepatitis (8). Our research aimed to determine if there is any association between SEN-V/TTV co-infection in HBV or HCV hepatitis patients and severity of liver disease.

Materials and Methods:

Subjects: The current study incorporate 100 blood samples gathered from 50 patients with hepatitis B or C from the Gastroenterology and Hepatology Teaching Hospital. Also, 50 blood samples were gathered from apparently healthy blood donors from the Blood Donation Center in Al Imamein Al Kadhimein Medical City, Baghdad, Iraq from November, 2017 to March, 2018. Blood was drawn from all patient and control subsequent to getting written consent. This study was confirmed by Ethical Committee at Al-Nahrain University, Collage of Medicine according to (MMM/23) document in 27/12/2017.

Samples preparation: Blood samples gathered from hepatitis patients and apparently healthy controls by venipuncture. Five (5) ml blood samples were drawn in sterile gel tubes then allowed to clot in (25) ˚C for one hr. After that they centrifuged at 3,000 rpm for 10 min and aliquot to three vials and stored at (-44) ˚C, each set of the three vials was given an identical code representing each individual serum specimen.

Molecular detection of SEN-V DNA:

Viral DNA extraction: After thawing of serum samples, viral DNA was extracted using Viral Nucleic Acid Extraction Kit II (Cat. #VR00, Geneaid, Taiwan) according to manufacturer's instructions.

SEN virus DNA Amplification: DNA amplification reactions were carried out for 100 samples by nested conventional PCR according to Hosseini *et al.* (2016) with major modifications to optimize the results (5). The first cycle took 5 min at 95 ˚C to

activate the enzymes. It involved 35 repeated cycles with every cycle consisted of 3 steps: denaturation of DNA template for 30 sec at 95˚C, annealing for 45 sec at 60˚C, and extension for 45 sec at 72˚C. At the end of these cycles, a final extension was done for 5 min at 72˚C. The same steps were applied in second cycle of nested-PCR, which was performed to amplify 1µl of the PCR products of the first PCR cycle. For visualization, 10 µl of PCR products were subjected to electrophoresis in (1%) agarose gel (Bio Basic, Canada) in the presence of ethidium bromide and visualized under UV transilluminator. The band size was assessed by direct comparison with a 100-bp DNA ladder. The sample is considered to be positive for SEN-V DNA if one band at 349 bp DNA fragment is observed after the first PCR round for all SEN-V genotypes, and a 124 bp band for SENV-H or 198 bp band for SENV-D after the second PCR round. Positive and negative control groups were run with each reaction.

Serological detection of TTV Ag: Qualitative detection of TTV Ag was carried out for 93 samples by enzyme-linked immunosorbent assay (ELISA) (Abbexa, England) according manufacturer's instructions. The optical density (OD) for the patient and control serum were calculated by comparing with the Cut off value according to the following equation: Cut off value = OD of negative control + (0.15). If the OD of samples ≥ Cut off, the test samples were considered positive. Otherwise, if the OD of samples < Cut off, the test samples were considered negative.

Biochemical investigations: Aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured by automated clinical chemistry analyzer (Dimension X pand, Germany) using serum sample for all subjects. This method is an in vitro diagnostic test intended for the quantitive determination of (ALT & AST) activity in human serum. The normal ranges were obtained from the manual of the kit (Normal values: AST =15-37 U/L, ALT =12-78 U/L).

Statistical Analysis: Data were analyzed using statistical packages for social sciences (SPSS) - version 19 (Chicago, IL, USA). For numerical data, mean ± standard deviation (S.D.) were calculated. Whereas, categorical data presented as count and percentage. Comparisons of demographic and clinical data between groups were made using Chi-square test (X^2-test) for categorical variables and independent sample t-test for mean differences. Odds ratio were calculated as estimates of the relative risk to test for any significant association between the outcome and the exposure. A P value <0.05 was taken as threshold of statistical significance.

Results

This case/control study included 50 patients with hepatitis type B or C infection, their mean age was 36.20±13.4 S.D. year and 50 apparently healthy blood donors as control, their mean age was 35.22±9.8 S.D. year. Thirteen (26%) out of 50 patients were infected with hepatitis type C, while 37 (74%) out of 50 patients were infected with hepatitis type B. Regarding sex distribution, male were 24 (48%) and female 26 (52%) in hepatitis patients, while in apparently healthy blood donors male represented 47 (94%) and female only 3 (6%). The liver function test parameters i.e. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were (50.9 ±49.7 and 52.6±56.5, respectively) in patients which higher than in control group (16 .0 ±6.0 and 22.96 ±7.7), (P>0.05).

SEN-V DNA was detected in 21 out of 50 (42.0%) patients by conventional PCR, while only 10 out of 50 (20%) of healthy blood donors were found to be SEN-V DNA positive, chi-square test showed that

there was a statistical significant association between the SEN-V DNA detection and the category of studied population (X^2=5.657, P<0.05), Table (1). For assessing odds ratio odds ratio were calculated as estimates of the relative risk to test for any significant association between presences versus absence of SEN DNA. Patient group is 2.897 times as likely to be SEN-V DNA positive as control group [Odds Ratio (OR) = 2.897].

Table (1): Occurrence of SEN-V DNA among cases and controls

SEN-V DNA status	Category		Total (%)	Statistic
	Patient group (n=50)	Control group (n=50)		
Positive	21(42.0%)	10 (20.0%)	31 (31%)	X^2=5.657 0.017
Negative	29 (58.0%)	40 (80.0%)	69 (69%)	
Total	50 (100.0%)	50 (100.0%)	100(100%)	
(OR, 2.897; 95% CI, 1.19-7.07)				

Torque Teno virus (TTV) infection was found to be higher in-patient group than in control group. TTV was detected in 37 (74%) of 50 hepatitis patients, while in apparently healthy blood donors, the frequency of TTV was 10 (23.26%) out of 43 which is statistically significant (P=0.000). Odd ratio (OR) was calculated as estimates of the relative risk to test for any significant association between presence versus absence of TTV Ag in case/control group. Case is 9.392 times as likely to be TTV Ag positive as control group (OR = 9.392), Table (2).

Table (2): Occurrence of TTV Ag among cases and controls.

TTV	Category		Total	Statistic
	Patient group (n=50)	Control group (n=43)		
Positive	37(74%)	10(23.26%)	47(50.54%)	X^2=23.81 *P=0.000*
Negative	13(26%)	33(76.74%)	46(49.46%)	
Total	50(100%)	43(100%)	93(100.0%)	
(OR, 9.392; 95% CI, 3.637-24.252)				

Chi-square test showed that there was no significant association between proportions of SEN-V and TTV in study population, as shown in table (3).

Table (3): Association between SEN-V and TTV in study population.

TTV	SEN		Total	*P* value
	Positive	Negative		
Positive	15(48.4%)	32(51.6%)	47(50.5%)	X^2=0.086
Negative	16(51.6%)	30(48.4%)	46(49.5%)	*P*=0.769
Total	31(100.0%)	62(100.0%)	93(100.0) %	

Co-infection with SEN-V and TTV was detected in (4.65%) among 43 blood donors and in (26%) among 50 viral hepatitis patients ($P<0.05$), Patients are 7.203 times as likely to be co-infected with SEN-V and TTV as blood donors (OR=7.203) as shown in table (4).

Table (4): Occurrence of SEN/TTV co-infection among patient and control groups.

SENV/TTV co-infection	Category		Total	Statistic
	Patient group (n=50)	Control group (n=43)		
Presence	13(26%)	2(4.65%)	15(16.13%)	X^2=7.78
Absence	37(74%)	41(95.35%)	78(83.87%)	*P*=0.005
Total	50(100%)	43(100%)	93(100.0%)	
(OR ,7.203;95% CI,1.523-34.058)				

Table (5) is showing the biochemical parameters i.e. liver enzymes (ALT and AST). A comparison was made between SENV infection alone and SENV/TTV co-infection as regarding liver enzymes ALT and AST, (34.44±27.792 versus 46.33±57.48) for ALT and (49.44±54.067 versus 42.07±41.897) for AST, the difference wasn't statistically significant ($P>0.05$). In addition, our previous studies (data not shown) (12, 13) found that ALT and AST did not significantly differ between SENV-positive and negative individuals among blood donors and hepatitis patient. However, regarding ALT measure, these previous studies showed a statistically significant difference between TTV-positive and negative groups which indicated that the presence of this virus could increase the severity of liver damage in hepatitis patients.

Table (5): Comparison between SEN-V infection and SENV/TTV co-infection in all study groups as regards to liver enzymes.

Biochemical tests	SENV alone	SENV/TT co-infection	Statistic
ALT(U/l)	34.44±27.792	46.33±57.48	t=0.741
AST(U/l)	49.44±54.067	42.07±41.897	t=-0.422

For additional characterization, the risk factors such as sex, history of blood transfusion, medical procedure and tattooing were evaluated. This study appeared that there was no statistically significant association between SENV positive alone and SENV/TTV co-infected

individuals and risk factors under study, Table (6).

Table (6): Comparison between SEN-V infection alone and SENV/TTV co-infection in study individuals as regards risk factors.

Risk factors		Total	SEN-V only	SENV/TTV co-infection	Statistic
Age		----------	36.19±11.80	33.60±9.59	t=0.68 *P=0.510*
Sex	**Female**	8 (25.8%)	4 (25.0%)	4 (26.7%)	X^2=0.011 *P=0.916*
	Male	23 (74.2%)	12 (75.0%)	11 (73.3%)	
	Total	31 (100.0%)	16 (100.0%)	15 (100.0%)	
History of blood transfusion	**Presence**	2 (6.5%)	1 (6.3%)	1 (6.7%)	X^2=0.416 *P=0.519*
	Absence	29 (93.5%)	15 (93.7%)	14 (93.3%)	
	Total	31 (100.0%)	16(100.0%)	15 (100.0%)	
History of surgery	**Presence**	10 (32.3%)	6 (37.5%)	4 (26.7%)	X^2=0.416 *P=0.519*
	Absence	21 (67.7%)	10 (62.5%)	11 (73.3%)	
	Total	31 (100.0%)	16 (100.0)	15 (100.0%)	
History of Tattooing	**Presence**	6 (19.4%)	2 (12.5%)	4 (26.7%)	X^2=0.955 *P=0.318*
	Absence	25 (80.6%)	14 (87.5%)	11 (73.3%)	
	Total	31 (100.0%)	16(100.0%)	15 (100.0%)	

Discussion:

The present case/control study indicated that the frequency of SEN-V and TTV in patients with hepatitis B or C was higher than in apparently healthy blood donors, as shown in Table (1) and Table (2), which was compatible with others (4,9). The association between TTV and SEN-V infections was analyzed and the results suggested that SEN-V infects both apparently healthy blood donors and liver disease patients independent of the occurrence of TTV, Table (3). In addition, TTV and SEN-V co-infection had been detected in hepatitis patient and healthy blood donors with exposure rate (26%) and (4.65%), respectively as shown in table (4). In other reports, the prevalence of TTV in patients with liver disease was reported to be 47% in Japan and, 27% in America and 21% of United Kingdom. The high frequency of these infections in patients with hepatitis B or C might be because of contaminated blood products and/or contaminated equipment items. Moreover, persistent blood transfusion and liver diseases have more noteworthy presentation to a hazard such as viral infections (4,9).

The mean ALT and AST was not statistically significant among individuals infected with SEN-V only compared to those co-infected with SEN-V and TTV (P>0.05) table (5). So, SEN-V infections alone or TTV/SEN-V co-infections had no biochemical evidence of significant liver damage in both hepatitis patients and control group. This is comparable to another previous study conducted by Kao *et al.* who showed that Torque Teno virus infection does not affect the disease nature in patients with hepatitis type B or C (3,14). In contrast, many studies have been reported that both viruses SEN-V and Torque Teno virus are correlated to high liver enzyme levels in patients with liver diseases, hemophilia, aplastic anemia, thalassemic patient and patients on

maintains hemodialysis after blood transfusion (4,8,15). Taking into consideration these results, SEN-V and/or TTV infection appears not to be related to the prompting or strengthen hepatic damage, which hence proposes that the routin screening test for TTV and SEN-V isn't essential in blood donation sitting.

Some risk factors, for example, sex, age, history of liver illness and history of blood transfusion have been assessed with SEN-V infection alone and SENV/TTV co-confection. The results have indicated that there is no association had observed between these viruses regarding, age, sex, history of blood transfusion, surgery and tattoo according to table (6). Another study conducted in diverse countries and individuals in considering clinical background showed similar results (16,17). In conclusion, the role of SEN-V and TTV in the pathogenesis of hepatitis may possibly decreased. On this point, the present study is in agreement with Mushahwar *et al.*, who found that SEN virus is related to Torque Teno virus and both of them are considered entirely as endosymbionts because of long history of mutual adaptation (18). SEN-V and TTV infection rates were higher in patients with liver disease than in apparently health blood donors suggested that blood transfusion may be a basic route for SEN-V and TTV transmission. But these viruses were also identified in healthy population whom have no history of blood transfusion suggests that it may be transmitted by ways other than blood transfusion (17).

Ethics Committee Approval: Ethics committee approval for this study was received from the Al-Nahrain College of Medicine Ethics Committee (Decision No: MMM/23, Decision Date: December 27, 2017).

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Conflict of Interest: The authors have no competing interests.

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