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ORIGINAL ARTICLE

The Frequency of Sen Virus and Torque Teno Virus (TTV) among Hemodialysis Patients

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Abstract

Background: The use of hemodialysis for end-stage renal disease has increased patient life span significantly. Infectious diseases caused by viruses that are commonly found in the liver include hepatitis C virus (HCV), SEN virus (SENV), and the Teno virus (TTV). This work aimed to find out the frequency of SENV & TTV infections and to correlate their viral load with their clinical condition among hemodialysis patients in Zagazig University Hospitals.

Methods: In a case-control study we included 44 patients divided into 2 groups; 22 hemodialysis patients attending to hemodialysis unit and 22 apparently healthy individuals served as control group. Detection of anti HCV antibodies & anti HBsAg was done by ELISA. Detection of SENV DNA and TTV as done by nested PCR analysis.

Results: Statistically significant differences were found between the studied groups regarding prevalence of SEN D, SEN H, coinfection SEN D and H, TTV and combined SEN/TTV genotypes (p=0.048, 0.021, 0.048, 0.026, and 0.021 respectively). SEN D, SEN H, TTV prevailed in 22.7%, 45.5% and 50% of patients on HD. Combined SEN V D and H prevailed in 22.7% while 45.5% of HD patients had coinfection with SENV and TTV. Also, statistically significant differences were revealed between the studied groups regarding prevalence of combined SEN H/D and TTV genotypes among hemodialysis patients and comorbid hypertension, diabetes ALT, AST, and hemodialysis duration (p=0.004, <0.001, 0.002,0.002 and 0.008 respectively).

Conclusions: We found a significantly higher prevalence of SEN virus D, SEN virus H, TTV, and concurrent infections involving combinations of these viruses among hemodialysis patients compared to healthy controls. The viral infections associated strongly with comorbid conditions like hypertension, diabetes, and liver enzyme abnormalities as well as longer duration on hemodialysis.

Keywords: Torque Teno Virus, Sen Virus, Hemodialysis

INTRODUCTION

Hemodialysis has greatly extended the life expectancy of cases who had end-stage renal disease. It also increases the risk of infections for hemodialysis patients, especially those caused by blood-borne viruses, which are a major cause of death and morbidity for this population [1]. Common viruses that cause liver disease include hepatitis C virus (HCV), hepatitis B virus (HBV), Teno virus (TTV) as well as SEN virus (SENV) among others [2].

Genome research indicated that hepatitis G virus (HGV) and TT virus were the main culprits in cases of hepatitis with no clear cause, indicating that it was not an A-E hepatitis. In modern times, SENV has a worldwide occurrence that varies by region. Non-A-G hepatitis has a new possible causative

agent, and this viral agent is the most recent to be suggested [3].

A recently identified virus, the SEN virus, has been suggested as a non-A to E hepatitis virus. Similar to TTV in remote relation, this virus has 3900 nucleotides of circular, single-stranded DNA [4].

Parentally transmitted viral diseases, including HIV, hepatitis B and C, and others, can infect hemodialysis patients. The fact that therapeutic operations often include bleeding and blood transfusions, combined with the fact that HD materials used in various medical institutes are not entirely disposable, puts them at a significant risk of contracting a blood-borne viral infection [5]. Because it is present in a broad variety of human samples, such as breast milk, synovial fluid, faeces, bile juices, and saliva, there are alternative routes of transmission besides blood transfusions, which is why populations with a history of blood transfusions tend to have a higher frequency of TTV [6]. To determine whether transfusion or dialysis is linked to an increased risk of TTV infection, it is important to study the prevalence of TTV in healthy patients [7].

The present work aimed to find out the frequency of SENV & TTV infections and to correlate their viral load with their clinical condition among hemodialysis patients in Zagazig University Hospitals.

METHODS

Subjects

In a case-control study, we conducted this research on cases attending the clinical pathology department in Zagazig University hospitals during the period from February 2023 to September 2023.

Written informed consents were obtained from all participants after an explanation of the procedure and medical research. The study was carried out after the approval of the Institutional Review Board (IRB), with approval number (#10670/26-4-2023) The research was conducted as per the World Medical Association's Code of Ethics (Helsinki Declaration) for human research.

This study was carried out on 44 subjects; 22 hemodialysis patients at the hemodialysis unit, in the internal medicine Department and 22 healthy volunteers age and sex matched.

Group 1: 22 hemodialysis patients attending to hemodialysis unit including 13 females with age range from 32 to 79 years old and 9 males with their ages ranging from 16 to 85 years old.

Group 2: 22 apparently healthy individuals serve as control group, including 10 females range from 42 to 68 years old and 12 males with their ages range from 35 to 65 years old.

Cases with the following criteria were included; adult patients with end stage renal disease (ESRD) undergoing hemodialysis (HD), who were age and sex matched with control group. Exclusion criteria: Patients having history or current diagnosis of HIV positive testing, autoimmune hepatitis, patients using hepatotoxic drugs or with history of alcohol abuse and patients refusing to enroll in the study.

Methods

Full clinical assessment which includes complete history taking (Name, age, sex, any existing medical disease (as DM and HTN), current medication, times of blood transfusions, duration of hemodialysis, history of previous surgery), clinical examination (by urology specialists).

Laboratory tests were performed including: Complete blood picture by Sysmex Xn-1000,Liver function test by Cobas 8000 (c702 module) (Rochez, Germany). Kidney function test measured by Cobas 8000(c702 module) (Roche, Germany). Fasting blood glucose using hexokinase method spectrophotometry on Cobas 8000 (c702 module) (Roche, Germany) was done.

Detection of anti HCV antibodies & anti HBsAg was done by ELISA.

Collection of blood samples

Under strict aseptic conditions, ten millilitres of venous blood were drawn from each subject. Three portions were taken from the samples following serum separation: Following the instructions provided by the manufacturer, the first aliquot was utilised to determine the ALT and AST levels using the Cobas 8000 (c702 module) (Roche, Siemens. Germany). Following the instructions provided by the manufacturer, the second aliquot was utilised to detect anti-HBsAg and anti-HCV antibodies in the serum using the Cobas 8000 (c602 module) (Roche, Siemens. Germany). Senov-D/H and TTV DNA detection by nested PCR were performed on the third aliquot, which was kept at -20˚C.

Extraction of DNA

Following the manufacturer's instructions, DNA was isolated from serum using a Gene JET Viral DNA and RNA Purification Kit from Thermo Scientific. The DNA was then kept at -20˚C.

Nested PCR for Detection of SENV DNA

Through the use of nested PCR, the partial ORF1 genes of SENV-D and SENV-H were amplified using thermal cycler ID: Applied Biosystems™ Veriti™ 96-Well Fast Thermal Cycler. For every 20µL reaction, the following components were

added according to the first round, primers, and PCR conditions listed in Table 1,10 microliters of DreamTaq Green PCR Master Mix (2X), 1 microliter of SEN-V-D/H forward primer and 1 microliter of SEN-V-D/H reverse primer, 5 microliters of DNA template, and 3 microliters of nuclease-free waterEach 20µL reaction for the second round of PCR amplification requires the following: 10µL of DreamTaq Green PCR Master Mix (2X), 1.0 µL of forward primer for SEN-V-D (20 pmol), 1.0 µL of reverse primer for SEN-V-D (20 pmol), 2µL of the 1st run PCR product, and 6µL of nuclease-free water with specific forward and reverse primers for SENV-D or SENV-H, following the same PCR cycle conditions as illustrated in Table 1 [8].

Semi nested PCR for Detection of TTV

In the initial round, for every 20µL reaction, the ORF1 gene of TTV was amplified. 10 microliters of DreamTaq Green PCR Master Mix (2X), 1 microliter of TTV forward primer (20 pmol), 1 microliter of TTV reverse primer (20 pmol), 5 microliters of DNA template, and 3 microliters of nuclease-free water. To conduct the second round of polymerase chain reaction (PCR), each 20µL reaction needs the following: 10 µL of DreamTaq Green PCR Master Mix (2X), 1.0 µL of forward primer for TTV (20 pmol), 1.0 µL of reverse primer for TTV (20 pmol), 2µL of the product from the first run of PCR, and 6µL of nuclease-free water. On examine the PCR results, ethidium bromide staining was applied to Agarose gel powder (Boehringer Mannheim, Germany), detection was conducted using agarose gel electrophoresis (Figure 1) [9].

Statistical analysis

The data was statistically analysed using IBM SPSS, version 25.0. (IBM Corporation, Armonk, New York). The Shapiro-Whitney U test was used to ensure that the data followed a normal distribution. The qualitative data was shown using relative percentages and frequencies. The stated difference between qualitative variables was calculated using the chi-square test ($\chi 2$) and Fisher exact. Parametric data was presented as mean ± SD (Standard deviation), whereas non-parametric data was presented as median and range. Parametric variables were compared using an independent T-test, whereas non-parametric variables were compared using a Mann Whitney test.

RESULTS

Non-significant differences were found between the studied groups regarding gender or age, while prevalence of diabetes, hypertension and hepatitis C, ALT and AST were significantly higher among the case group ($p<0.001$, 0.003, <0.001, <0.001, <0.001 respectively) (Table 2).

There were statistically significant differences between the studied groups regarding prevalence of SEN D, SEN H, coinfection SEN D and H, TTV and combined SEN/TTV genotypes ($p=0.048$, 0.021, 0.048, 0.026 and 0.021 respectively). SEN D, SEN H, TTV prevailed in 22.7%, 45.5% and 50% of patients on HD. Combined SEN V D and H prevailed in 22.7% while 45.5% of HD patients had coinfection with SENV and TTV, statistically significant differences were found between the studied groups regarding prevalence of SEN D among hemodialysis patients, prevalence of TTV genotypes among all of diabetes, hypertension, ALT, AST and hemodialysis duration ($p= 0.035$, 0.04, 0.005, 0.003, and 0.01 respectively), also statistically significant differences were revealed between the studied groups regarding prevalence of SEN H among hemodialysis patients and prevalence of TTV genotypes, hypertension, diabetes, ALT, AST and SEN D genotypes ($p= 0.001$, 0.004, 0.01, <0.001, <0.001 and 0.01 respectively) (Table 3).

There were statistically significant differences between the studied groups regarding prevalence of combined SEN H/D among hemodialysis patients and prevalence of TTV genotypes and ALT, AST, hypertension and hemodialysis duration ($p=0.035$, <0.001, <0.001, 0.04, and 0.01 respectively) (Table 4).

Statistically significant differences were found between the studied groups regarding prevalence of TTV among hemodialysis patients and prevalence of TTV genotypes and diabetes, hypertension, ALT, AST, SEN H and hemodialysis duration ($p<0.001$, <0.001, 0.013, 0.013, 0.001, and <0.001 respectively) (Table 5).

Statistically significant differences were revealed between the studied groups regarding prevalence of combined SEN H/D and TTV genotypes among hemodialysis patients and comorbid hypertension, ALT, AST, diabetes and hemodialysis duration ($p=0.004$, 0.002, 0.002, <0.001, and 0.004 respectively) (Table 6).

TTV significantly increased risk of SEN H genotypes among studied hemodialysis patients by 45 folds, hypertension and diabetes increased risk of combined SEN/TTV among hemodialysis patients by 14.968 and 27.253 folds respectively (Supplementary Table 1).

Table (1): Primers and PCR conditions for genes detected in the study

Gene	Primer Sequence (5′ –3′)	PCR cycle conditions
ORF1 for SENVD/H (1st Round)	**F:** AI-1F: 5'-TWC YCM AAC GAC CAGCTA GAC CT-3' (W= A or T, Y= C or T, M= A or C) **R:** AI-1R: 5'- GTT TGT GGT GAG CAG AAC GGA-3'	1st round: initial denaturation at 95°C for 3 min followed by 40 cycles (95˚C for 60 seconds, 55˚C for 30 seconds and 72˚C for 60 seconds for each cycle) with final extension time for 10 minutes at 72˚C in the thermocycler
SEN virus-D (2nd Round)	**F:** D-1148F: 5'- CTA AGC AGC CCT AAC ACT CAT CCA G-3' **R:** AI-1R: 5'- GTT TGT GGT GAG CAG AAC GGA-3'	2nd round: 25 cycles (95˚C for 20 seconds, 65˚C for 30seconds and 72˚C for 30 seconds) for both SENV-D and SENV-H.
SEN virus-H: (2nd Round)	**F:** H-1020F: 5'- TTT GGC TGC ACC TTC TGG TT-3' **R:** H-1138R: 5'- AGA AAT GAT GGG TGA GTG TTA GGG-3'	
ORF1 for TTV virus: (1st Round)	TTV-F: 5'- ACA GAC AGA GGA GAA GGC AAC ATG-3' TTV-R: 5'- CTG GCA TTT TA CCA TTT CCA AAG TT -3'	1st round: initial denaturation at 95°C for 9 min, followed by 55 cycles of denaturation at 95°C for 20 sec, annealing at 55°C for 20 sec and at 72° for 30 sec. The program was followed by a final extension at 72°C for 5 min.
TTV virus: (2nd Round)	**TTV-FF**: 5'- GGCAACATGTTATG GATAGACTGG-3' **TTV-R**: 5'- CTG GCA TTT TA CCA TTT CCA AAG TT -3'	2nd round: 25 cycles of the same conditions

Table (2): Demographic data, Comorbidities and laboratory data of studied groups

	Case group N=22 (%)	Control group N=22 (%)	χ^2	p
Gender: **Male** **Female**	10 (45.5%) 12 (54.5%)	12 (54.5%) 10 (45.5%)	0.364	0.546
	Mean ± SD	**Mean ± SD**	t	p
Age (year)	56.6 ± 12.4	54.0 ± 8.99	0.766	0.448
Diabetes: Present Absent	12 (54.5%) 10 (45.5%)	1 (4.5%) 21 (95.5%)	13.211	<0.001**
Hypertension: Present Absent	11 (50%) 11 (50%)	2 (9.1%) 20 (90.9%)	8.844	0.003*
Previous surgery/blood transfusion Present Absent	7 (31.8%) 15 (68.2%)	2 (9.1%) 20 (90.9%)	3.492	0.062
Hepatitis B Positive Negative	2 (9.1%) 20 (90.9%)	0 (0%) 22 (100%)	Fisher	0.488
Hepatitis C Positive Negative	10 (45.5%) 12 (54.5%)	0 (0%) 22 (100%)	12.941	<0.001**
ALT Normal Elevated	9 (40.9%) 13 (59.1%)	22 (100%) 0 (0%)	18.452	<0.001**
Median(IQR)	66(30.75 – 89)	23(18 – 27)	Z(-4.753)	<0.001**
AST Normal Elevated	9 (40.9%) 13 (59.1%)	22 (100%) 0 (0%)	18.452	<0.001**
Median(IQR)	58(24 – 79.5)	23(20.5 – 27)	Z(-3.186)	0.001**

χ^2 Chi square test t independent sample t test, Z Mann Whitney test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table (3): Comparison between the studied groups regarding prevalence of SEN and TTV genotypes and Relation between presence of SEN D and SEN H with studied parameters among HD group

	Case group N=22 (%)	**Control group** N=22 (%)	χ^2	**P**	**COR (95% CI)**
SEN D: **Positive** **Negative**	5 (22.7%) 17 (77.3%)	0 (0%) 22 (100%)	Fisher	0.048*	∞
SEN H: **Positive** **Negative**	10 (45.5%) 12 (54.5%)	3 (13.6%) 19 (86.4%)	5.35	0.021*	5.28(1.2 – 23.16)*
TTV: **Positive** **Negative**	11 (50%) 11 (50%)	4 (18.2%) 18 (81.8%)	4.956	0.026*	4.5(1.15 – 17.68)*
SEN VD/H: **Positive** **Negative**	5 (22.7%) 17 (77.3%)	0 (0%) 22 (100%)	Fisher	0.048*	∞
Combined SENV/TTV **Positive** **Negative**	10 (45.5%) 12 (54.5%)	3 (13.6%) 19 (84.6%)	5.35	0.021*	5.28(1.2 – 23.16)*

Relation between presence of SEN D and studied parameters among HD group				
	Positive SEN D **N=5(%)**	Negative SEN D **N=17(%)**	χ^2	p
Gender: **Male** **Female**	3 (60%) 2 (40%	10 (58.8%) 7 (41.2%)	Fisher	>0.999
Age (mean ± SD)	58.8 ± 13.03	55.82 ± 12.54	0.463¥	0.648
Diabetes: **Present** **Absent**	5 (100%) 0 (0%)	6 (35.3%) 11 (64.6%)	Fisher	0.035*
Hypertension: **Present** **Absent**	5(100%) 0 (0%)	7 (42.2%) 10 (57.8%)	Fisher	0.04*
Previous surgery/blood transfusion **Present** **Absent**	2 (40%) 3 (60%)	5 (29.4%) 12 (70.6%)	Fisher	>0.999
Duration: **>12 months** **<12 months**	5 (100%) 0 (0%)	5 (29.4%) 12 (70.6%)	Fisher	0.01*
Hepatitis B **Positive (n=2)** **Negative**	0 (0%) 5 (100%)	2 (11.8%) 15 (88.2%)	Fisher	>0.999
Hepatitis C **Positive (n=10)** **Negative**	3 (60%) 2 (40%)	7 (42.2%) 10 (57.8%)	Fisher	0.624
TTV **Positive** **Negative**	5 (100%) 0 (0%)	6 (35.3%) 11 (64.7%)	Fisher	0.035*
	Median(IQR)	**Median(IQR)**	Z	P
ALT	394(151.5 –489)	57(31 – 117.5)	-2.704	0.005*
AST	139.5(123 – 385)	26.5(20.6 – 51)	-2.782	0.003*

Relation between presence of SEN H and studied parameters among HD group				
	Positive SEN H **N=10(%)**	Negative SEN H **N=12(%)**	χ^2	p
Gender: **Male** **Female**	 5 (50%) 5 (50%)	 5 (41.7%) 7 (58.3%)	0.153	0.696
Age (mean ± SD)	59.9 ± 10.75	53.67 ± 13.4	1.186¥	0.25
Diabetes: **Present** **Absent**	 8 (80%) 2 (20%)	 3 (25%) 9 (75%)	6.6	0.01*
Hypertension: **Present** **Absent**	 9 (90%) 1 (10%)	 3 (25%) 9 (75%)	Fisher	0.004*
Previous surgery/blood transfusion **Present** **Absent**	 2 (20%) 8 (80%)	 5 (41.7%) 7 (58.3%)	Fisher	0.381
Duration: **>12 months0** **<12 months**	 7 (70%) 3 (30%)	 3 (25%) 9 (75%)	Fisher	0.084
Hepatitis B **Positive (n=2)** **Negative**	 1 (10%) 9 (90%)	 1 (8.3%) 11 (91.7%)	Fisher	>0.999
Hepatitis C **Positive (n=10)** **Negative**	 5 (50%) 5 (50%)	 5 (41.7%) 7 (58.3%)	0.153	0.696
TTV **Positive (n=11)** **Negative**	 10 (100%) 0 (0%)	 1 (8.3%) 11 (91.7%)	Fisher	0.001**
SEN D **Positive (n=5)** **Negative**	 5 (50%) 5 (50%)	 0 (0%) 12 (100%)	Fisher	0.01*
	Median(IQR)	**Median(IQR)**	Z	P
ALT	198.5(119 –486.25)	37.5(28 – 79.5)	-3.365	<0.001**
AST	188(113.5 –426.75)	33.5(23 – 75.75)	-3.43	<0.001**

χ^2 Chi square test COR crude odds ratio CI Confidence interval *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table (4): Relation between presence of coinfection SEN D, H and studied parameters among HD group

	Positive SEN D/H **N=5(%)**	Negative SEN D/H **N=17(%)**	χ^2	**p**
Gender: **Male** **Female**	 2 (40%) 3 (60%)	 8 (47.1%) 9 (52.9%)	Fisher	>0.999
Age (mean ± SD)	55.8 ± 13.81	56.71 ± 12.4	-0.14¥	0.89
Diabetes: **Present** **Absent**	 4 (80%) 1 (20%)	 7 (41.2%) 10 (58.8%)	Fisher	0.311
Hypertension: **Present** **Absent**	 5(100%) 0 (0%)	 7 (41.2%) 10 (58.8%)	Fisher	0.04*
Previous surgery/blood transfusion **Present** **Absent**	 1 (20%) 4 (80%)	 6 (35.3%) 11 (64.7%)	Fisher	>0.999

Duration: **>12 months (** **<12 months**	5 (100%) 0 (0%)	5 (29.4%) 12 (70.6%)	Fisher	0.01*
Hepatitis B **Positive (n=2)** **Negative**	0 (0%) 5 (100%)	2 (11.8%) 15 (88.2%)	Fisher	>0.999
Hepatitis C **Positive (n=10)** **Negative**	3 (60%) 2 (40%)	7 (41.2%) 10 (58.3%)	Fisher	0.624
TTV **Positive (n=11)** **Negative**	5 (100%) 0 (0%)	6 (35.3%) 11 (64.7%)	Fisher	0.035*
	Median(IQR)	**Median(IQR)**	Z	P
ALT	439.5(220.75 – 491.0)	65.5(31 – 132.5)	-2.74	0.005*
AST	156(94 – 400)	46(27 – 75.5)	-2.725	0.003*

χ^2 Chi square test Z Mann Whitney test ¥independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table (5): Relation between presence of TTV and studied parameters among HD group

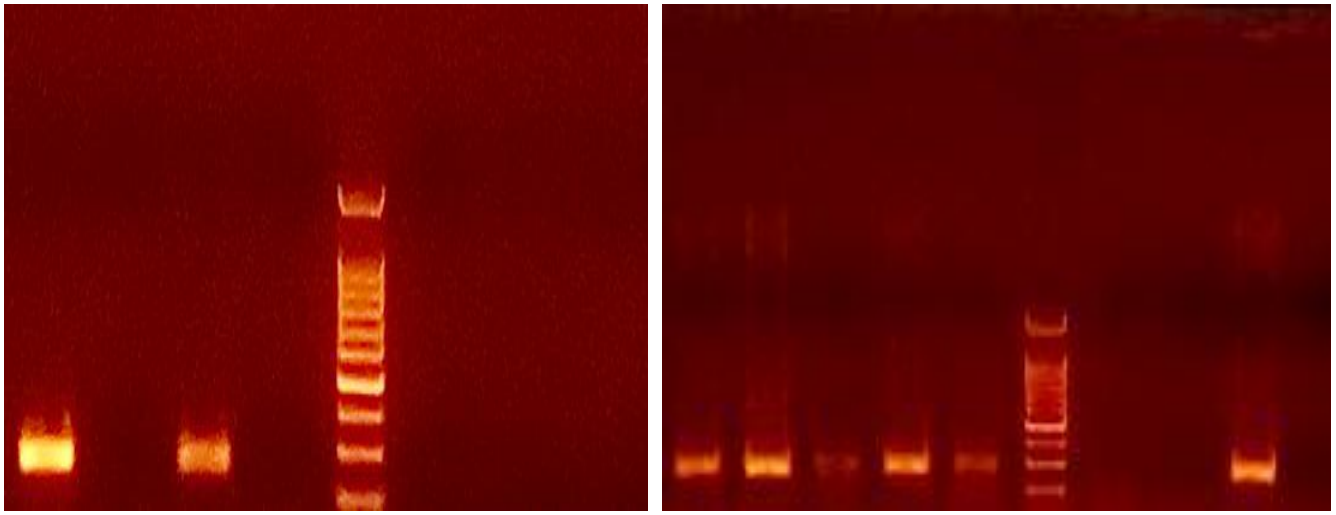
	Positive TTV **N=11(%)**	**Negative TTV** **N=11(%)**	**χ^2**	**p**
Gender: **Male** **Female**	6 (54.5%) 5 (45.5%)	4 (36.4%) 7 (63.6%)	0.733	0.392
Age (mean ± SD)	60.5 ± 10.13	52.45 ± 13.57	1.585¥	0.129
Diabetes: **Present** **Absent**	10 (90.9%) 1 (9.1%)	1 (9.1%) 10 (90.9%)	Fisher	<0.001**
Hypertension: **Present** **Absent**	10 (90.9%) 1 (9.1%)	2 (18.2%) 8 (81.8%)	11.733	<0.001**
Previous surgery/blood transfusion **Present** **Absent**	3 (27.3%) 8 (72.7%)	4 (36.4%) 7 (63.6%)	Fisher	>0.999
Duration: **<12 months** **>12 months**	9 (81.8%) 2 (18.2%)	1 (9.1%) 10 (90.9%)	11.733	<0.001**
Hepatitis B **Positive (n=2)** **Negative**	0 (0%) 11(100%)	2 (18.2%) 9 (81.8%)	Fisher	0.476
Hepatitis C **Positive (n=10)** **Negative**	5 (45.5%) 6 (54.5%)	5 (45.5%) 6 (54.5%)	0	>0.999
SEN D **Positive (n=5)** **Negative**	5 (45.5%) 6 (54.5%)	0 (0%) 11 (100%)	Fisher	0.035*
SEN H **Positive (n=10)** **Negative**	10 (81.8%) 1 (18.2%)	0 (9.1%) 11 (90.9%)	11.733	0.001**
	Median(IQR)	**Median(IQR)**	Z	P
ALT	86(27.8 – 163)	57(31 – 87)	-2.431	0.013*
AST	26(21 – 74)	67(27 – 81)	-2.937	0.016*

χ^2 Chi square test Z Mann Whitney test ¥ independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

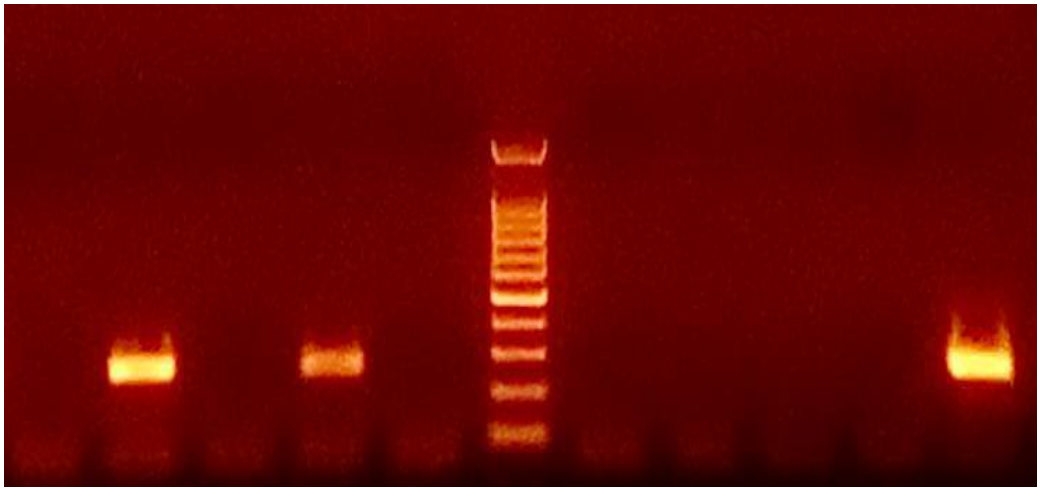
Table (6): Relation between presence of combined SEN and TTV and studied parameters among HD group

	Positive TTV+SEN N=10(%)	**Negative TTV/SEN N=12(%)**	χ^2	p
Gender:				
Male	5 (50%)	5 (41.7%)	0.153	0.696
Female	5 (50%)	7 (58.3%)		
Age (mean ± SD)	60.5 ± 10.68	53.17 ± 13.17	1.414¥	0.173
Diabetes:				
Present	9 (90%)	2 (16.7%)	11.733	<0.001**
Absent	1 (10%)	10 (83.3%)		
Hypertension:				
Present	9 (90%)	3 (25%)	Fisher	0.004*
Absent	1 (10%)	9 (75%)		
Previous surgery/blood transfusion				
Present	2 (20%)	5 (41.7%)	Fisher	0.381
Absent	8 (80%)	7 (58.3%)		
Duration:				
>12 months	8 (80%)	2 (16.7%)	Fisher	0.008*
<12 months	2 (20%)	10 (83.3%)		
Hepatitis B				
Positive (n=2)	1 (10%)	1 (8.3%)	Fisher	>0.999
Negative	9(90%)	11 (91.7%)		
Hepatitis C				
Positive (n=10)	5 (50%)	5 (41.7%)	0.153	0.696
Negative	5 (50%)	7 (58.3%)		
	Median(IQR)	**Median(IQR)**	Z	P
ALT	198.5(119 – 486.25)	50(28 – 83.75)	-3.002	0.002*
AST	446(213.25 –531.75)	59(26.75 – 59)	-3.034	0.002*

χ^2 Chi square test Z Mann Whitney test *p<0.05 is statistically significant ¥ independent sample t test **p≤0.001 is statistically highly significant



(A) (B)



(C)

Figure (1): Agarose gel electrophoresis of PCR products showing (A): positive SENV-D, (B): Positive SENV-H and (C): Positive TTV

DISCUSSION

Hemodialysis treatments increase the likelihood of hepatitis C virus transmission. The hepatitis B virus, the SEN virus, and the torque teno virus are all examples of such viruses (TTV). It is debatable whether or not the latter two viruses cause liver illness [10].

A tiny circular DNA virus that is neither enveloped nor helical, SENV is a member of the family Circoviridae. Parenteral and vertical routes of transmission are included. We know of nine distinct strains, numbered A through I. Due to their association with non-A-E hepatitis, strains D and H have received the lion's share of clinical attention. Both healthy people and hepatitis patients have tested positive for SENV strains [11].

TTV belongs to the Circoviridae family of viruses and is characterised by having a circular DNA structure with just one strand. So far, five separate genogroups have been recognized (groups 1 to 5). TTV can be transmitted through the intestinal, vertical, or parenteral routes; it is found in bodily fluids and organs [12].

In the present study study, non-significant differences were found between the studied groups regarding gender or age. Also, Elaskary et al. [12] studied 314 participants, 80 of whom were hemodialysis patients (85 males and 72 females, mean age 47.62±10.71) and 57 healthy blood donors (90 males and 67 females, mean age 47.01±11.07) who served as a control group. There was no significant difference ($p < 0.05$) in the age and sex distribution between the two groups.

In the present study, there were statistically significant differences between the studied groups regarding prevalence of hepatitis C as they were significantly higher among case group (45.5% vs 0%). While, there was no statistically non-significant difference between the studied groups regarding hepatitis B (9.1% vs 0%).

This slightly agrees with Elaskary et al. [12] who reported that, in comparison to the control group, which tested negative for both viruses, the hemodialysis group exhibited a significantly greater percentage of HBV (29.9 percent) and HCV (34.4 percent) ($p<0.001$).

This was somewhat in line with the findings of Abd ElHady et al. [13], who found that 27.3% of hemodialysis patients and 29.1% of the negative control group had HCV and HBV, respectively.

It was also somewhat concordant with the findings of Dai et al. [14], who found that hepatitis C virus (HCV) prevalence was 24.2% in hemodialysis patients and 7.1% in negative blood donors.

This was somewhat in line with what Abdel Hady et al. found. According to the results reported in [15], there was no statistically significant difference between the hemodialysis patients' and blood donors' detection rates of HCV and HBV, at 25.3% and 30.6% of the hemodialysis patients and negative controls, respectively. The dissimilarities in the features of the control group might explain this while HCV was found to be extremely prevalent among the hemodialysis group in another research [16, 17].

In the present study, a statistically significant difference was found between the studied groups regarding prevalence of diabetes (54.5%) and hypertension (50%) (all were significantly higher among case group).

Among the many causes of renal failure and the subsequent need for dialysis, diabetes ranks high. Additionally, hypertension is common among patients with chronic renal disease [18].

The majority of the hemodialysis patients (42.7 percent) and the control group (3.8 percent and 3.2 percent, respectively) complained of diabetes and hypertension, according to Elaskary et al. [12], with a statistically significant difference ($p< 0.001$).

This was in line with the findings of Lea et al. [19], who discovered that 50.1% of patients with end-stage renal disease were diabetic and 27.0% were hypertensive.

The majority of hemodialysis patients were men (75%), while a small percentage were hypertensive (19%) and diabetic (19%), according to Elfaki et al. [18]. Among haemodialysis patients, Gorsane et al. [20] found a 90% greater prevalence of hypertension.

In the present study, a statistically significant difference was found between the studied groups regarding ALT and AST (all were significantly higher among case group). About 59% of patients within case group had elevated ALT and AST.

According to Abdel Hady et al. [15], there was a highly significant difference ($P < 0.01$) in AST and a statistically significant difference ($P < 0.05$) in ALT between the tested groups. This could mean that hemodialysis patients exhibited increased rates of SENV and TTV affection in addition to higher rates of HBV and HCV affection.

In the present study, statistically significant differences were revealed between the studied groups regarding prevalence of SEN D, SEN H, coinfection SEN D and H, TTV and combined SEN/TTV genotypes. SEN D, SEN H, TTV prevailed in 22.7%, 45.5% and 50% of patients on HD. Combined SEN V D and H prevailed in 22.7% while 45.5% of HD patients had coinfection with SENV and TTV. While in control group, no patient had SEN D or coinfection SEN D/H, Three patients had TTV+ SEN H.

An earlier research found that 89.1% of the hemodialysis patients and 16% of the control patients tested positive for SEN virus [13]. Abd ElHady et al. [13] found SENV-H in 65.5% of hemodialysis patients and SENV-D in 23.6% of controls; the corresponding percentages for the two groups were 12% and 4%, respectively.

Among hemodialysis patients and controls, 68% and 38% of SENV were found by Kobayashi et al. [17] and Kao et al. [21], respectively.

In terms of TTV, research by Wahid & Saadoon [22] and Ali et al. [23] found a detection rate of 40.9% in hemodialysis patients and 38.7% in controls, respectively.

The total prevalence of SENV with its two genotypes was shown to be significantly different in hemodialysis patients (27.3% vs. 5.8%), controls (46.5 vs. 18.3%), and hemodialysis patients (61.6 vs. 23.3%), according to Dai et al. [14]. When comparing the prevalence of SENV-H between hemodialysis patients (12.8%) and controls (16.8%), Schroter et al. [24] found no statistically significant differences.

This agreed with the findings of Afkari et al. [25], who found that 19.33% of hemodialysis patients and 9.33% of healthy blood donors had coinfections with SENV and TTV, respectively.

A recent study found that while 4.65% of healthy blood donors did not have a coinfection between SENV and TTV, 26% of hepatic patients did [26].

Some TTV genotypes have been linked to specific diseases and conditions, such as renal pathology, acute respiratory disorders, arthritis, laryngeal cancer, post transfusion hepatitis, and others.

In the present study, statistically significant difference was found between the studied groups regarding prevalence of combined SEN H/D among hemodialysis patients and prevalence of TTV genotypes.

The researchers Elaskary et al. [12] discovered a strong correlation between TTV and SENV-D and SENV-H [13]. In the control group, a highly significant correlation was found between TTV and SENV-H coinfection 4 (40 percent), SENV-D coinfection 3 (42.9 percent), and SENV-D/H coinfection 1 (non-significant) (50 percent).

Also, according to Pirouzi et al. [11], there is a stronger correlation between TTV and SENV-H. They found that 43.33 percent of HIV patients had coinfection of SENV and TTV, with 32.66% showing signs of SENV-H and 23.33% showing signs of SENV-D. There was a coinfection of TTV and SEV in 21.33 percent of blood donors.

The present study revealed non-significant differences in the prevalence of various viral infections between hepatitis B/C positive and negative hemodialysis patients. Specifically, the rates of SEN virus D (SENV-D) genotypes, SENV-H genotypes, combined SENV-D/H infection, TTV infection alone, and concurrent triple infection with SENV-D/H and TTV were statistically similar between hemodialysis patients regardless of hepatitis B or C status. These non-significant

associations were observed across all the viral infection types and combinations evaluated. Therefore, these data indicate that co-infection with hepatitis B or C neither raises nor lowers the risk of acquisition with SENV or TTV in this population. The lack of interaction between these viruses implies that immunosuppression from renal failure and hemodialysis underlies transmission, rather than hepatic viral interference effects.

The results were in agreement with those of Hosseini et al. [32], who found that compared to healthy individuals (90.5 percent), the frequency of SENV and its two genotypes was considerably lower ($P < 0.05$) in hepatitis B and hepatitis C patients (56%).

Although the correlation between TTV and any kind of hepatic disease has not been investigated as of yet, Magu et al. [33] found elevated amounts of TTV DNA in liver tissue and the presence of TTV infection in posttransfusion hepatitis.

When looking for evidence of hepatitis C virus (HCV) seropositivity, Hassuna et al. [34] discovered it in 65% of patients, but HBsAg was detected in just 2%. (2 percent). There is no evidence of a correlation between TTV and either HCV or HBV in terms of co-infection status. This could be because TTV can be transmitted by non-parenteral channels, even though all three viruses were transmitted from parents.

The present study revealed a significantly higher prevalence of SEN virus D (SEN-D) genotypes among hemodialysis patients compared to controls, associating it with TTV infection, hypertension, diabetes, and liver enzymes. Similarly, SEN-H genotypes were more prevalent in hemodialysia patients and linked to the same factors plus SEN-D positivity. Though not statistically significant, over 12 months on hemodialysis trended towards hugely increased risk of dual SEN-D/H infection. Combined SEN-D/H infection associated significantly with hypertension, liver enzymes, and dialysis duration. TTV infection itself was more common in hemodialysis patients, connecting with the aforementioned variables except age/gender/previous surgeries/transfusions. Lack of diabetes offered protection from TTV. Finally, concurrent triple infection with SEN-D/H and TTV tied strongly to comorbid hypertension, diabetes, liver test abnormalities and longer hemodialysis duration.

Consistent with previous studies by Ali et al. [23], Pirouzi et al. [11], and Kobayashi et al. [17], their findings demonstrated that there was no significant relationship between TTV and SENV and age, sex, HBsAg, HCV antibody, diabetes mellitus type 2, hypertension, or a history of prior surgery. However, there was a strong correlation between SENV and TTV infection and factors such as the length of time on hemodialysis, a history of blood transfusions, and liver enzymes (AST and ALT).

Contradict what Abd ElHady et al. found in relation to AST and ALT, two liver enzymes. Contrary to what Schröter et al. found, [13] found that hemodialysis patients infected with SENV had no changes to their AST or ALT levels, suggesting that SENV had little to no liver pathogenicity. [24] findings.

According to the findings of Khudair et al. [26], there was no statistically significant difference between subjects infected with SENV and those infected with both TTV and SENV in terms of the increase of AST and ALT.

Hassuna et al. [34] reported that, Aside from age, no other demographic variable (sex or duration of dialysis) was linked to a high incidence of TTV. The intriguing discovery was that TTV viremia was more common in younger subjects, which may be attributable to the virus's potential clearance with age. An increased risk of tuberculosis infection (TTV) is associated with prolonged dialysis treatments, which may be attributable to both the longer duration of exposure and the worsening immune-deficiency that dialysis patients experience over time. In addition, dialysis procedures significantly add to the risk of infection in these individuals [7].

Hassuna et al. [34] reported that, AST and ALT levels showed no statistically significant difference across all TTV groups, supporting the theory that TTV is a commensal virus and that only specific genotypes and genogroups cause liver disease [35].

Limitations of the current study includes: Small sample size from a single center limits generalizability, we were unable to determine causality due to observational case control study design, we did not evaluate impact of viral infections on clinical outcomes like mortality, and there was possible selection bias in the hemodialysis group from recruiting only symptomatic patients. Larger multi-center studies on risk factors and complications of SENV/TTV coinfections are needed to explore viral transmission routes in dialysis units to identify protective measures.

Conclusions

Our study found a significantly higher prevalence of SEN virus D, SEN virus H, TTV, and concurrent

infections involving combinations of these viruses among hemodialysis patients compared to healthy controls. The viral infections associated strongly with comorbid conditions like hypertension, diabetes, and liver enzyme abnormalities as well as longer duration on hemodialysis. The lack of significant difference in viral infection rates between hepatitis B/C positive and negative dialysis patients suggests these viruses prevail independently.

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Supplementary Table (1) Binary regression analysis showing factors significantly associated with SEN H infections and factors significantly associated with combined TTV and SEN infections

Factors significantly associated with SEN H infections					
				95% C.I.	
	β	**p**	**AOR**	**Lower**	**Upper**
Positive TTV	3.807	0.004*	45	3.46	584.34
Factors significantly associated with combined TTV and SEN infections					
				95% C.I.	
	β	**p**	**AOR**	**Lower**	**Upper**
Hypertension	2.706	0.075	14.968	0.762	293.93
Diabetes	3.315	0.024*	27.523	1.542	491.38

AOR adjusted odds ratio CI Confidence interval *p<0.05 is statistically significant

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