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Molecular and Phylogenetic Detection of Torque Teno Virus (TTV) Among Hemodialysis Patients: A Single Center Study

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Abstract

Background Patients on long-term hemodialysis are at a higher risk of contracting blood transmitted infections because of their weakened immune systems and frequent interaction with blood, blood products, equipment, and contaminated surfaces. This making them an important population to study the clinical and epidemiological consequences of newly discovered infections like torque teno virus (TTV).

Objective To investigate the frequency of TTV in patients undergoing hemodialysis by molecular method and to find out any association with risk factors and liver function tests.

Methods This cross-sectional study conducted on (100) patients whom attending Hemodialysis Unit at Al-Imamein Al-Kadhimein Medical City in Baghdad, Iraq. The sample of the study consist of (58) males and (42) females, their mean age is (49.97±4.97 SD) years, (50) with viral hepatitis, while (50) without viral hepatitis, for a period from November 2020 to March 2021. Nested polymerase chain reaction (PCR) was used to detect TTV-DNA. TTV genogroup was determined by Sanger sequencing and phylogenetic tree construction.

Results TTV DNA was detected in 81% (81 out of 100) of hemodialysis patients, respectively. However, no significant association was found between demographic data, clinical characteristics and risk factors with TTV infection.

Conclusion This study showed high prevalence of TTV in hemodialysis patients but didn't play a role in liver injury among these patients. Also, based on phylogenetic analysis of the untranslated region (UTR), genogroup-3 was found to be the most prevalent in hemodialysis patients.

Keywords Torque teno virus, hemodialysis, risk factors, liver function, sequencing, phylogenetic tree

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List of abbreviations: ALP = Alkaline phosphatase, ALT = Alanine transaminase, AST = Aspartate aminotransferase, HD = Hemodialysis patients, PCR = Polymerase chain reaction, TSB = Total serum bilirubin, TTV = Torque teno virus, UTR = Untranslated region

Introduction

In addition to well-known (A to E) hepatitis viruses, there is growing evidence that newer hepatitis viruses may exist and have a role in this disease [1-3]. Torque teno virus (TTV) was first discovered in Japan 1997 from serum of patients with post transfusion non-A to G hepatitis who developed raised alanine aminotransferase level (ALT) [4]. This virus was designated as (T.T) initially from the first patient from whom the virus was isolated [5]. It has a circular DNA genome with a negative strand, but a significantly large genome size [6]. At first, TTV got classified as part of the family circoviridae, genus anellovirus [7]. It was later classified as a member of the Anelloviridae

family [8]. The TTV genome sequence is very diverse in nature and many genetic variants have been detected by using high conserved untranslated region (UTR) as a specific primer for the detection of DNA. Interestingly, the coding regions of TTV are less conserved than the UTR. For instance, the coding region of open reading frame 1 (ORF 1) contains three hypervariable regions (HVRs) in tandem. Variability within ORF 1, which is believed to code for the TTV capsid protein, may be crucial to evasion of the host immune system [9]. According to phylogenetic analysis, TTVs belong to a broad ancestral tree of five important genogroups, and the first and second of them are most prevalent worldwide [10].

TTV is thought to be transmitted by blood transfusion, and its frequency is mainly associated with populations with a history of blood product transfusion [11,12]. Moreover, there are two groups highly associated with TTV virus, these groups are patients undergoing hemodialysis (HD) and intravenous drug users [13]. TTV has been detected in the sera of persons who have not had a blood transfusion, indicating that additional routes of transmission may exist [14]. Virus detection by genogroup-specific polymerase chain reaction (PCR) in serum samples from various geographic regions (China, Canada, Korea, Spain, France, Thailand and the USA) has shown a prevalence ranging from 33% to 100% [15]. The TTV DNA frequency in the Italian HD unit (41.7%) was greater than in the healthy population (10.7%) [16]. While in Indian dialysis patients the universality of TTV DNA was (83%) [17]. In 1999, Gallian et al. showed that the general seroprevalence of TTV was 28 % in HD patients in France [18], on the other hand Kheradpezhou et al. suggested that the seroprevalence was 9.3% in HD patients [19]. There are many reasons behind the higher prevalence in developing countries, which include socioeconomic factors, bad infection control measures and the higher prevalence of the virus among general population (20).

This study aimed to determine the frequency of TTV DNA in hemodialysis patients, as well as, estimation of demographic and clinical data. Also, to determine TTV genogroup prevalence in those patients.

Methods

Subjects

Study conducted on one hundred patients whom attending Hemodialysis Unit of Al-Imamein Al-Kadhimein Medical City to undergo hemodialysis from November 2020 to March 2021. A questionnaire was used to collect data from patients which include sex and age of patients, hepatitis B virus (HBV) and hepatitis C virus (HCV) status, underlying medical condition, history of kidney transplant, previous surgery, marital status, duration of hemodialysis, blood transfusion and number of blood transfusions. In addition, information of liver function test was obtained at the time of study from patient's laboratory report. The ethical approval of the research project was provided by the Institutional Review Board (IRB) at Al-Nahrain University on 20th September 2020 (No. 20200977).

Specimens' collection

Five (5) ml whole blood was drawn from each patient prior to starting hemodialysis in sterile gel tubes and allowed to clot at 25°C for one hour, then centrifuged at 3000 rpm for 10 minutes, serum samples were divided into aliquots in sterile eppindrof tubes which then stored at -20°C until be used.

Isolation of viral DNA

To detect the presence of TTV nucleic acids, DNA was extracted from 200 µl aliquots of serum using the viral nucleic acid extraction kit II (Geneaid, Taiwan) for viral nucleic acid purification from cell-free samples following the manufacturer instructions.

PCR detection of TTV DNA

For detection of TTV DNA, nested PCR specific for detection of sequences included in the UTR region (primers NG034/NG147, and NG133/NG132) as reported by Sarairah et al.

[21] and in the ORF-1 region (N22) (primers NG059, NG063 and NG061) according to Rinonce et al. [22]. The reason behind chosen two regions to amplify is that N22 region is better to the study the genetic variability, however, UTR is the most conserved region identified among strains [23]. The PCR reaction (25 µl) for the first and second rounds of PCR for the both reactions have been prepared by adding 5 µl of the template, 1.5 µM of each primer, 12.5 µl of one Taq PCR master mix (Biolabs, USA) and complete the final volume by adding 4.5 µl of nuclease-free water. Positive and negative controls were run with each round of PCR in order for the results to be considered valid under the conditions given. The thermal cycler settings were customized as those described by both Sarairah et al. and Cancela et al. [21,23] with modifications to optimize the result, as shown in the tables (1 & 2). The amplicon obtained in the first and second run of UTR region is equal to 143 and 110 nucleotides, respectively. Whereas for ORF1, it is equal to 286 and 271 nucleotides, respectively [22]. Both second amplicon region for UTR (110 bp) and ORF (271 bp) were observed in 3% agarose gel [21,24], as shown in the figure (1 & 2).

Table 1. Polymerase chain reaction program for both first and second round reaction using UTR region

Step	Temperature	Time	Cycles description	Cycles
1	94˚C	3 min	Initial denaturation	1
	94˚C	30 sec.	Denaturation	
2	60˚C	40 sec.	Annealing	35
	70˚C	30 sec.	Extension	
3	70˚C	5 min	Final extension	1

Table 2. Polymerase chain reaction program for both first and second round reaction using ORF1 region

Step	Temperature	Time	Cycles description	Cycles
1	94 ˚C	3 min.	Initial denaturation	1
	94 ˚C	30 sec.	denaturation	
2	55 ˚C	45 sec.	Annealing	40
	72 ˚C	45 sec.	Extension	
3	72 ˚C	7 min.	Final extension	1

Quality control

Positive and negative controls were run with each round of PCR in order for the results to be considered valid under the conditions given. Positive control was TTV-Ag positive samples by enzyme-linked immunosorbent assay (ELISA). While negative control was consisted of the reagents that used to prepare the PCR amplification mixture without TTV-DNA and treated like a sample.

Sanger sequencing and phylogenetic tree construction

The PCR amplicons of TTV-UTR region (110 bp) of 10 samples were commercially sequenced from their reverse termini following the guidelines manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). By comparing the obtained DNA sequences of the local samples with the

recovered DNA sequences of the national center for biotechnology information (NCBI) database, the virtual positions and other information of the recovered PCR were recognized. The detected variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server. After that, a traditional rectangular tree comprising the detected variant was created by the neighbor-joining method and annotated as a cladogram tree using the iTOL suit [25]. To know the exact identity of the genogroup of local TTV isolates, many references sequences of each TTV genogroup was represented, from genogroup-1 to genogroup-5.

Statistical analysis

Analysis of data was performed by using statistical package for social sciences (SPSS). Comparison is obtained by the use of Chi-square (χ2-test), whereas, numerical data presented as mean, standard deviation and evaluated by using independent sample T-test. Analysis of variance (ANOVA) was used to compare the means of more than two independent groups. The P-value same as or below 0.05 has been considered statistically significant, below 0.01 has been recommended highly significant, and above 0.05 was considered non-significant.

Results

Frequency of TTV based on 5-UTR A and ORF-1

One hundred (100) serum samples have been tested for the TTV DNA by nested PCR as described by Sarairah et al. and Cancela et al. [21,23]. The present study indicated that 81% of hemodialysis patients tested positive for TTV DNA for UTR region as illustrated in the figure (1). While only 1 out of 100 (1%) samples was positive for ORF-1 region as shown in the figure (2).

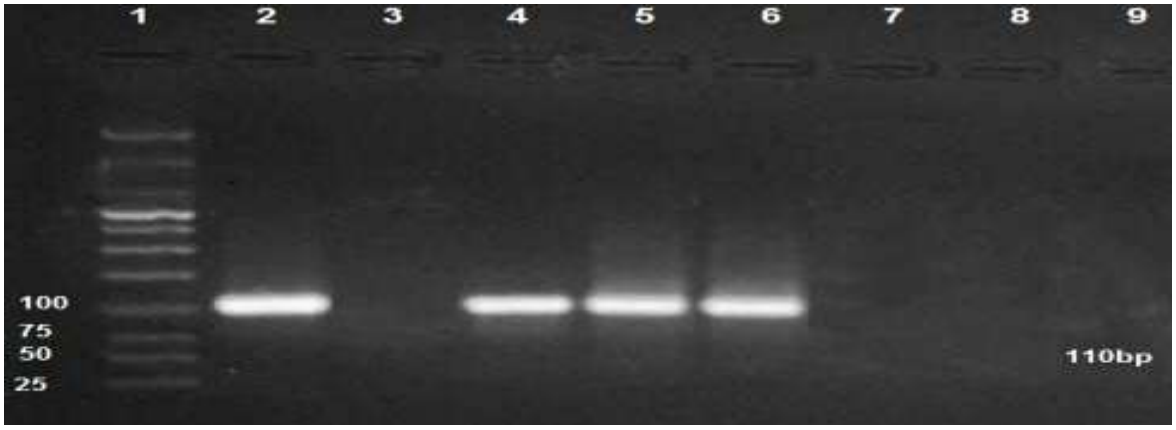

Figure 1. Gel-electrophoresis of second-round PCR products (5-UTR region) using 3% agarose in Tris-acetate-EDTA (TAE) buffer. Lane1, (25bp DNA marker), Lane2: positive control for amplification, Lane3: negative control for amplification, Lane 4,5,6: positive samples, and lanes 7,8,9: negative samples

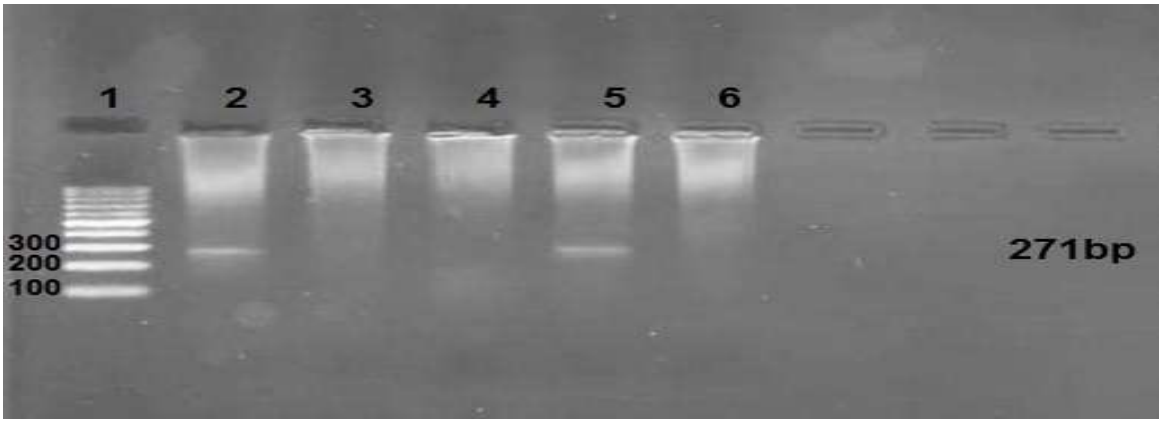

Figure 2. Gel-electrophoresis of second-round PCR products (ORF-1 region) using 3% agarose in TAE buffer. Lane1, (100bp DNA marker), Lane2: positive control for amplification, Lane3: negative control for amplification, Lane 4 and 6: negative samples, and Lane 5: positive sample

Demographic data, clinical characteristic and risk factors

This study showed no significant association between age, sex, diabetes mellitus (DM), hypertension (HT), history of kidney transplantation, history of previous surgery, marital status, hemodialysis duration, history of blood transfusion, number of blood transfusions, viral hepatitis and TTV infection.

Regarding co-infection with hepatitis, current study showed that 50 (50%) out of 100 hemodialysis patients were tested negative to hepatitis B virus (HBV) and/or hepatitis C virus (HCV) and 39 (78%) of them had TTV infection. While the remaining 50 (50%) out of 100 of hemodialysis patients had HCV &/or HBV. TTV was detected in 1 (50%) out of 2 of hemodialysis patients who had co-infected with HBV and 38 (84.4%) out of 45 of hemodialysis patients who had co-infected with HCV. In addition, three patients had triple infection with HBV, HCV and TTV. However, current study showed that there was no significant association between these viruses and TTV status, as shown in the table (3).

Table 3. Demographic data, clinical characteristic, risk factors and TTV status

Parameters		Total No. (%)	TTV- DNA Status (UTR region) Negative No. (%)	Positive No. (%)	*P*-value
Age (mean± SD) year		100 (100%)	48.53±14.10	50.31±15.23	0.634
Age groups (years)	<31	12 (12%)	2 (16.7%)	10 (83.3%)	0.439
	31-40	17 (17%)	3 (17.6%)	14 (82.4%)	
	41-50	17 (17%)	6 (35.3%)	11 (64.7%)	
	51-60	30 (30%)	5 (16.7%)	25 (83.3%)	
	> 60	24 (24%)	3 (12.5%)	21 (87.5%)	
Sex	Male	58 (58%)	14 (24.1%)	44 (75.9%)	0.124
	Female	42 (42%)	5 (11.9%)	37 (88.1%)	
DM	No	67 (67%)	14 (20.9%)	53 (79.1%)	0.491
	Yes	33 (33%)	5 (15.2%)	28 (84.8%)	
HT	No	12 (12%)	0 (0.0%)	12 (100%)	0.074
	Yes	88 (88%)	19 (21.6%)	69 (78.4%)	
History of kidney transplant	No	100 (100%)	19 (19.0%)	81 (81.0%)	–
	Yes	0 (0%)	0 (0.0%)	0 (0.0%)	
History of previous surgery	No	52 (52%)	10 (19.2%)	42 (80.8%)	0.951
	Yes	48 (48%)	9 (18.8%)	39 (81.2%)	
Marital status	No	13 (13%)	2 (15.4%)	11 (84.6%)	0.722
	Yes	87 (87%)	17 (19.5%)	70 (80.5%)	
Hemodialysis duration (year)	< 1	3 (3%)	2 (66.7%)	1 (33.3%)	0.103
	1-3	28 (28%)	7 (25%)	21 (75%)	
	3-5	23 (23%)	4 (17.4%)	19 (82.6%)	
	≥5	46 (46%)	6 (13%)	40 (87%)	
History of blood transfusion	No	34 (34%)	4 (11.8%)	30 (88.2%)	0.186
	Yes	66 (66%)	15 (22.7%)	51 (77.3%)	
No. of blood transfusions (time)	<1	44 (44%)	6 (13.6%)	38 (86.4%)	0.476
	1-4	38 (38%)	9 (23.7%)	29 (76.3%)	
	≥4	18 (18%)	4 (22.2%)	14 (77.8%)	
Virology	Negative	50 (50%)	11 (22%)	39 (78%)	0.444
	HBsAg	2 (2%)	1 (50%)	1 (50%)	0.259
	HCV-Ab	45 (45%)	7 (15.6%)	38 (84.4%)	0.427
	HBV & HCV	3 (3%)	0 (0.0%)	3 (100%)	0.394

DM: diabetes mellitus; HT: hypertension. HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus

Liver function tests

This study shows that there is no significant difference between TTV positive and TTV negative hemodialysis patients in the mean serum level of liver function enzymes such as alanine transaminase (ALT), aspartate Aminotransferase (AST), total serum bilirubin (TSB) and alkaline phosphatase (ALP), as shown in table (4).

Table 4. Serum level of liver enzymes and TTV DNA Status

*Biochemical test (Mean± SD)	TTV-DNA Status		*P*-value
	Negative	Positive	
ALT mg/dl	22.37±14.01	17.88±15.72	0.256
AST mg/dl	16.68±7.73	20.03±15.70	0.370
TSB mg/dl	0.41±0.22	0.35±0.18	0.252
ALP mg/dl	156.74±119.25	201.75±195.35	0.339

Normal value of liver enzymes: ALT= (10-49 mg/dl), AST= (<34 mg/dl), TSB= (0.3-1.2 mg/dl), ALP= (40-130 mg/dl)

DNA Sequencing of 5-UTR A amplicons

Alignments were done by NCBI-Blastn pairwise of the ten local TTV isolate with the most corresponding viral sequences from NCBI that indicated high similarity with variable TTV isolates from America, Europe, and Asia. Local TTV isolates were assigned as (S1 to S10), both S1 and S2 samples had high similarity with the isolate TTVMY02 from Malaysia (GenBank acc. no. MN116509.1). S3, S6, S7, and S8 showed a high similarity with the isolate TUS01 from USA (GenBank acc. no. AB017613.1). S4 and S9 samples showed high similarity with the isolates P10-1 and CK001, respectively which from American and Indian (GenBank acc. no. KT163893.1and KM596845.1). Both S5 and S10 samples were highly similar to the isolated TTV-HD24a and T520, respectively which from two German sources (GenBank acc. no. FR751506.1 and GU722347.1). The current result showed that six nucleic acid substitutions mutation and only one insertion mutation that distributed in the investigated fragments of the local isolate compared with reference sequences according to the figures (3 & 4). The S4 sample observed C48A, T45C and Gins43-44, the S5 sample observed T47G and A41G, while S9, S10 observed A30G and A29C, respectively.

Phylogenetic tree

In the present study, phylogenetic tree was generated using neighbor-joining method, which was based on the 50-UTR A region. This tree constructed by utilizing 22 NCBI relative reference sequences belonging to the significant five TTV genogroups (genogroup-1 to genogroup-5). Within this tree, 4 representatives TTV reference sequences for each of the genogroup 1, 2, 4, and 5 were incorporated. Meanwhile, 6 representatives for the genogroups 3 were incorporated due to its higher variability and availability among TTV. In addition, 10 local TTV isolates were incorporated within this tree.

It was found that the local isolates S1, S2, and S9 were incorporated within the clade of genogroup-2. It deserves to note that both S1 and S2 were equally suited in the vicinity of the KAV isolate (GenBank acc. no. AF435014). Meanwhile, the S9 sample was positioned in the vicinity of two TTV isolates, Kt-08 and Kt-010F. In addition, it was found that all the rest samples were incorporated within the clade of the genogroups-3.

Within the genogroups-3, these viral isolates were distributed into two main positions. It was found that the S3, S6, S7, and S8 were positioned in the vicinity of the TUS0 isolate. Likewise, the S3, S6, S7, and S8 were also respectively positioned in the vicinity to the isolate SENV-C of the same clade. Furthermore, it was found that S4, S10, and S5 were respectively suited in the vicinity to the isolate SANBAN, as shown in table (5) and figure (5).

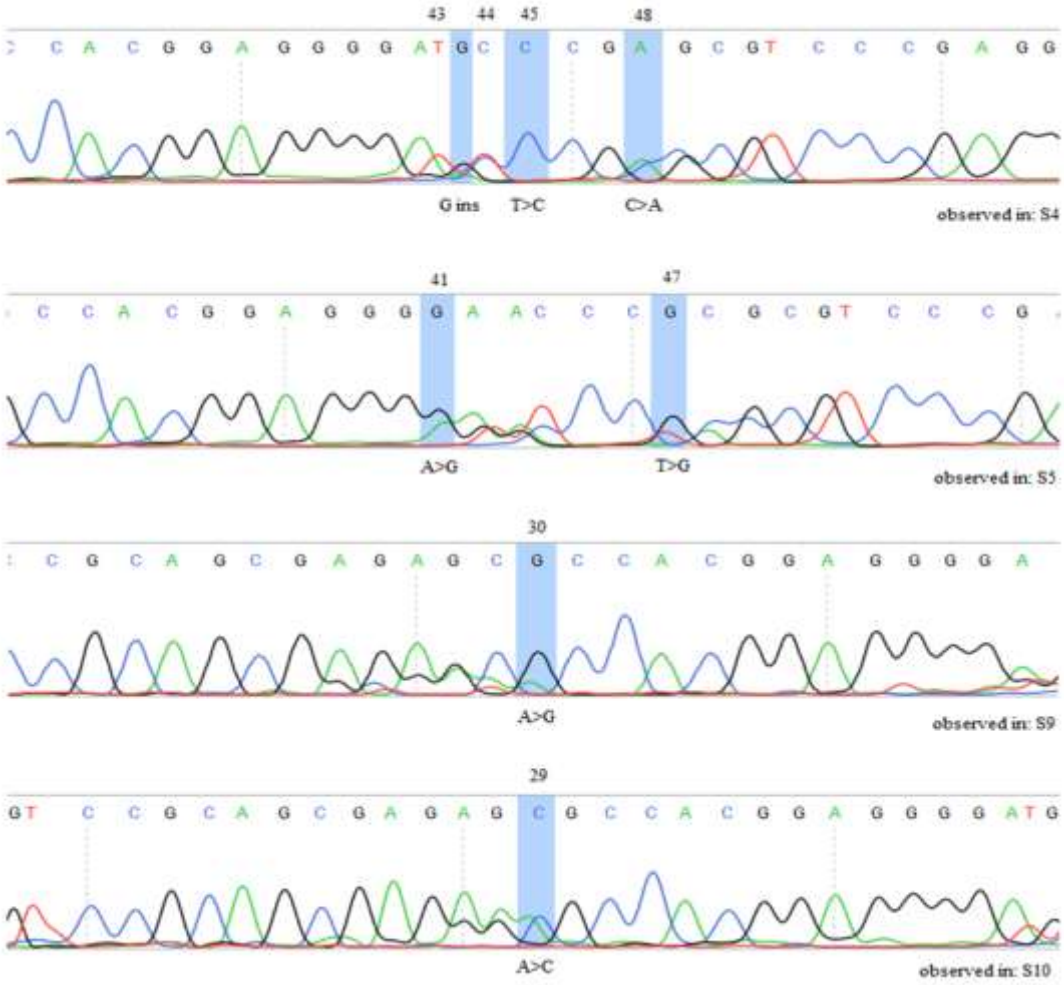

Figure 3. The chromatogram profile of the observed genetic variants of the UTR locus within the TTV viral isolates. The identified substitution mutations are highlighted according to their positions in the PCR amplicons. The letter "ins" refers to "insertion mutation", while the symbol ">" refers to "substitution" mutation. The letter "S" refers to the sample code

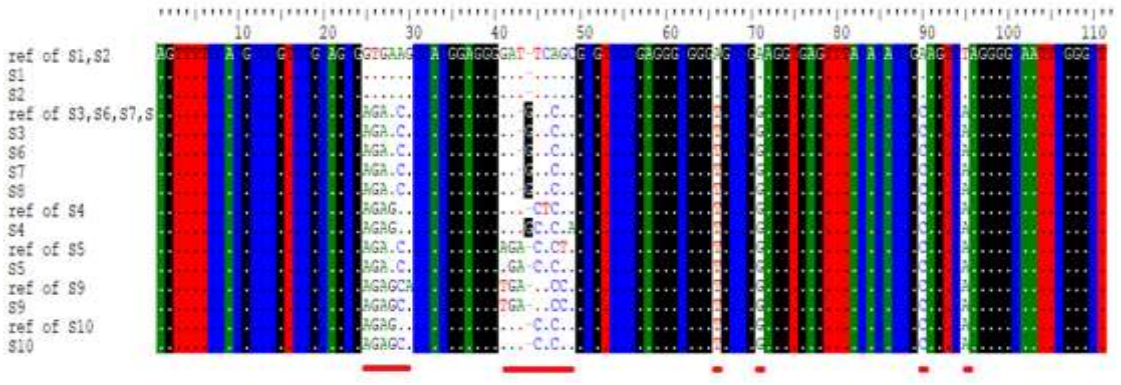

Figure 4. The determination of TTV DNA sequence variability regions in TTV DNA sequences via direct DNA alignment. the red arrows indicated the variable regions, while the colored boxes indicated the conserved regions. The symbol "ref" refers to the NCBI reference sequences, while "S" refers to sample code

Table 5. TTV reference isolates representative of the 5 major genomic groups

Isolate	GenBank acc. no.	Genogroup	Reference
JA20	AF122914.3	1	Erker et al., 1999 [26]
T3PB	AF247138.1	1	AbuOdeh et al., 2015 [27]
JA10	AF122919.1	1	Erker et al., 1999 [26]
JA4	AF122917.1	1	Erker et al., 1999 [26]
Kt-08	AB054647.1	2	AbuOdeh et al., 2015 [27]
Kt-010F	AB054648.1	2	AbuOdeh et al., 2015 [27]
ViPi04	DQ361268.1	2	Maggi et al., 2006 [28]
KAV	AF435014.1	2	Heller et al., 2001 [29]
SENV-D	AX025730.1	3	Okamoto et al., 2001 [30]
TJN01	AB028668.1	3	AbuOdeh et al., 2015 [27]
SENV-E	AX025761.1	3	Okamoto et al., 2001 [30]
SANBAN	AB025946.1	3	AbuOdeh et al., 2015[27]
SENV-C	AX025718.1	3	Okamoto et al., 2001[30]
TUS01	AB017613.1	3	Okamoto et al., 1999 [5]
TTVyon-LC011	AB038622.1	4	Takahashi et al., 2000 [31]
TTVyon-KC009	AB038621.1	4	Takahashi et al., 2000 [31]
TTVyon-KC197	AB038624.1	4	Okamoto et al., 2001 [30]
TTVyon-KC186	AB038623.1	4	Okamoto et al., 2001 [30]
BIS8-17	GU797360.1	5	Hussain et al., 2012 [32]
JT34F	NC_014076.1	5	AbuOdeh et al., 2015 [27]
CT44F	NC_014075.1	5	AbuOdeh et al., 2015 [27]
CT39F	AB064604.1	5	Peng et al., 2002 [33]

Discussion

TTV infection was found to be very common in people who suffered from idiopathic fulminant hepatitis and those suffering from cryptogenic chronic liver diseases including liver cirrhosis, chronic hepatitis and hepatocellular carcinoma [34]. On the other hand, researchers found that the frequency of TTV infection was the same in patients suffering from liver disease including assorted cases of non-B and non-C liver diseases, and also in few healthy individuals. Moreover, they settled that TTV does not cause harm to the liver [35]. In the present study, the prevalence of TTV-DNA was 81 out of 100 patients (81%) based on TTV 5-UTR A region amplification. While, only one was detectable by ORF-1 (N22 region) amplification. This results in agreement with Kenarkoohi et al. who found that TTV prevalence was 92% by using UTR primers and was 5% when using ORF primer [36]. One of the reasons for such results is that UTR primers is more conserved than N22 primers. Another reason could be that UTR PCR can detect more TTV genotypes than N22 PCR which has limited detection [37,38]. In addition, the failure to amplify the N22 region could be attributed to the extremely high variability observed in these strains, which indicated that they may contain new variants [23].

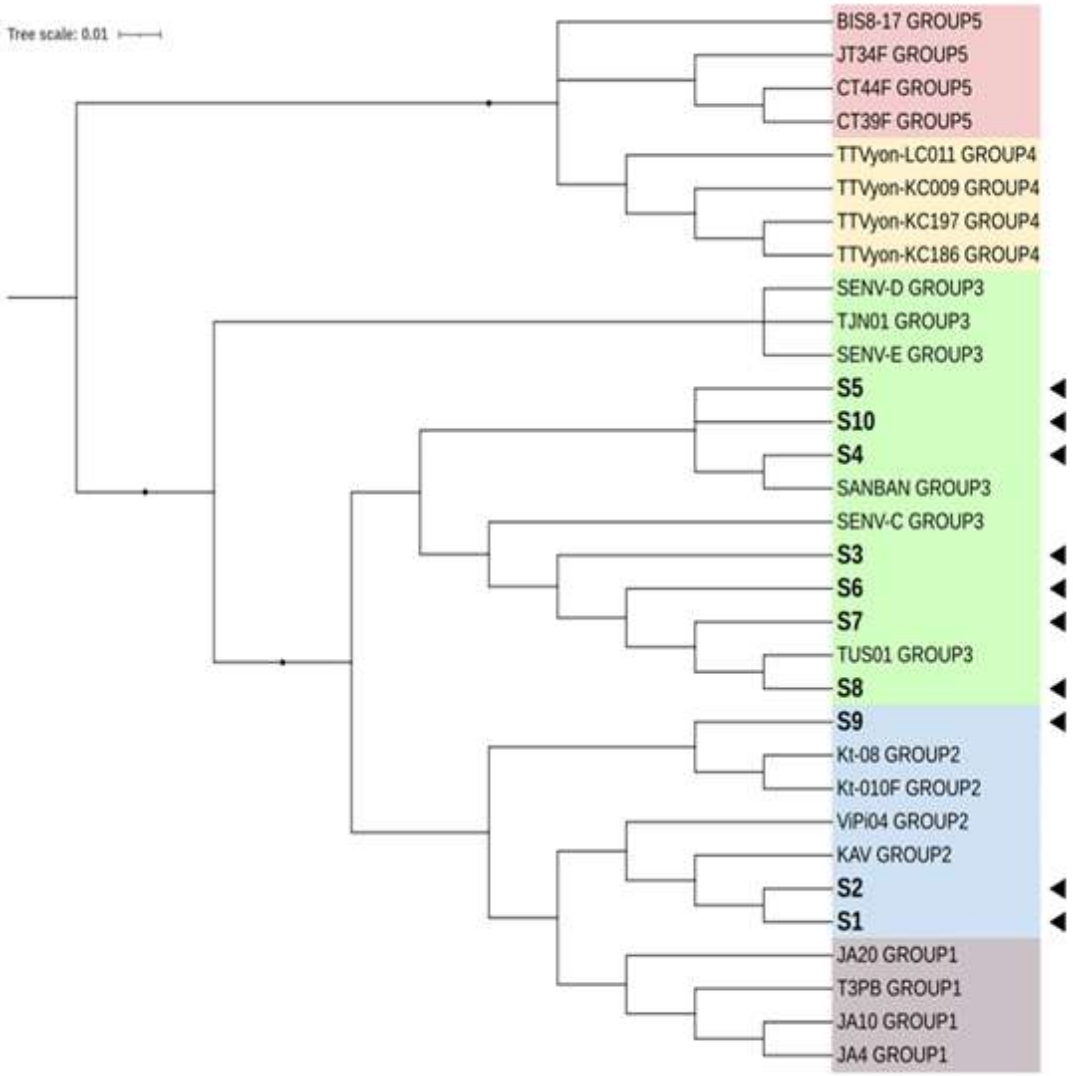

Figure 6. The comprehensive phylogenetic tree of genetic variants of the 5'-UTR locus within ten Torque teno viral isolates. The variably colored numbered refer to the specific genogroup incorporated. The scale of the left portion of the tree refers to the degree of phylogenetic positions among the tree categorized viral organisms. The symbol "S" refers to the code of the investigated samples

Among the current hemodialysis patients, 58 (58%) were males and 42 (42%) were females. Their mean age was (49.97±4.97 SD) years ranging between 14 and 80 years. Considering age of studied population, most of the TTV-DNA positive individuals were with mean age of (50.31±15.23) years according to the table (3). In the current study, there was no meaningful difference between the mean of age between TTV positive and negative HD patients which is close to what's mentioned by Irshad et al. [39]. On the other hand, disagree with Hassuna et al. who showed that there is a correlation between TTV viremia and age [40]. Regarding sex of studied patients, the higher rate (88.1%) of TTV DNA infection was in female rather than male (75.9%) with no significant association between sex and TTV DNA-status, as shown in table (3). These results are in accordance with Mohamed et al. (2017) who showed that the percentage of patients who were positive for TTV as (66.7%), out of

these patients, males were (3.7%) and females (6.1%) with no significant difference in TTV infection between males and females [41]. And these findings are disagreed with Takemoto et al. (2015) who showed that the majority of dialysis patients were males (55%) with a mean age of 53.8 years [42]. In addition, there is no significant statistical association between TTV and clinical characteristics or risk factors according to the table (3). These results are in agreement with Akbari et al. (2018) who observed that there is no significant relation between TTV infection and hypertension [43]. Another study also in accordance with the current findings done by Gallian et al. who found that the TTV prevalence in HD diabetic patients was not significantly higher than that detected in a diabetic patient without renal disease [18]. While, a study done by Spandole-Dinu et al. founded that there is a significant higher level of anelloviral DNA in type 2 diabetes mellitus (T2DM patients) than controls [44].

It is worthy to note that there is no one of the patients in this study underwent a kidney transplant so the relation between TTV infection and kidney transplantation is not known, because of the small sample size due to the fact that during the coronavirus pandemic, researchers were only able to analyze one hemodialysis center.

Regarding the surgical history, the present study showed that there was no noticeable significant association between the positivity of TTV infections and people who undergone surgery in agreement with Khudair et al. [1]. In addition, results showed no association between marital status and TTV infection in consistent with Yazici et al. who found that the TTV was not statistically significant between sexually transmitted risk groups and control group [38]. Patients in hemodialysis treatment are with major concern of infection [45,46]. This susceptibility is increased by their compromised clinical state like immune status and psychological condition especially long duration on HD as well as their extended and frequent exposure to a variety of potential risks, such as blood transfusion [45,47]. Considering the hemodialysis duration, the current study showed a non-significant difference between the TTV infections and the period of hemodialysis. This is near to other study done by Irshad et al. who indicated that TTV infection was not significantly associated with duration of hemodialysis [39]. Although nosocomial infection still may play an important role, but present study can't be ruled out that TTV had other transmission routes such as faeco-oral route, salivary droplet, sexual transition, breastfeeding [48,49]. While Jahromi et. al. reported a significant relation between TTV infection and the period of hemodialysis [50]. Importantly, there was no significant relation between the TTV infection and blood transfusion in hemodialysis patients in accordance with Irshad et al. study [39]. The use of erythropoietin to treat renal anemia resulted in a significant reduction in blood transfusions; however, infections in hemodialysis units can still occur in the absence of other parenteral risk factors [51,52].

The co-infection of TTV and HBV or HCV is common [2,53,54]. Although there was no significant association between hepatitis and TTV status in present study. However, the results showed, that 84.4% of HD patients who infected with HCV were TTV positive and 50% of them who had HBV were infected with TTV, table (3). This result in agreement with Najafimemar et al. who found that TTV-DNA was detected in 54% with chronic hepatitis B infection [55]. Also, this study is close to what's mentioned by Magu et al. who detected TTV DNA in 77% of HCV patients [42]. Furthermore, in Iraq, TTV-Ag was detected in 89 of the HBV-positive patients and in 30.8% of the HCV-positive patients [2]. The current data disagree with Chattopadhyay et al. who showed that among TTV-positive patients, HCV co-infection was absent [17]. Also, another study found the TTV-DNA in 7% of HBV positive patients [23]. In addition, three patients in current study had triple infection with HBV, HCV and TTV. These results are logic since these viruses share the parenteral route of transmission.

According to table (4), there were no significant difference between the level of liver function tests in TTV positive and negative HD patients. This suggests that TTV presence did

not cause severe damages to the liver. This is similar to study done by Irshad et al. who stated that the HD patients with TTV infection did not necessarily have liver dysfunction [56]. Another study by Fabrizi et al. suggested that there was a significant decrease in the concentration of ALT and AST in the hemodialysis patients compared to pre-dialysis patients with chronic renal failure [57]. This may be due to hemodialysis patients decreased secretion of Th-1 associated cytokine (INF-γ), but increased secretion of Th-2 associated cytokine (IL-10), resulting in immunological deficiency [49]. These results are clearly agreed with Lemon et al. who recommended that there are harmless viruses referred to as orphan viruses and are beneficial to the body in a way that they maintain homeostasis [58]. These viruses were isolated but not yet associated with any infection, so they are considered "simple guests". Although it may be difficult to attribute the term guest or endosymbiont to viral agents, but they have a characteristic responsible for altering the normal functioning of cells [59].

In the present study, the alignment of local TTV isolates sequences showed the presence of 99% to 100% of homology with the reference sequences from America, Europe and Asia. However, the result of local viral isolates (S1 to S10) showed the detection of six nucleic acid substitutions and only one insertion mutation according to the figures (3 & 4). Phylogenetic analysis showed that of the 10 sequences analysed, 7 genogrouped with genogroup 3 (S3-8, S10) and only 3 with genogroup 2 (S1-2, S9) as in the figure (5). Based on phylogenetic analysis, both S1 and S2 were completely identical (100%), this is may be due to acquiring the infection from the same source of infection. i.e., nosocomial transmission among hemodialysis patients. In addition, the reason for the tilt of the S9 sample toward both Kt-08 and Kt-010F isolates was attributed to the sequence variations observed in this sample.

The spread of viral strains with the same genotypes, could confirm the possibility of virus dissemination in the hemodialysis setting. The homogeneity of these genotypes reflects a nosocomial transmission [60].

In conclusion, the present study concluded a high frequency of TTV among Iraqi hemodialysis patients with prevalent genogroups-3, however, TTV may didn't seem play a role in liver injury as indicated by liver function tests and therefore at this time mandatory screening for TTV may not be recommended for hemodialysis units.

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Author contribution

All authors contributed to this manuscript. Dr. Al-Shuwaikh: design, interpreted and arranged this manuscript, Ali: performed all the laboratory work and implementation of this study as a part of her M.Sc. study, Dr. Manuti helps in clinical aspect and collection of samples.

Conflict of interest

There is no conflict of interest.

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