

Full Length Research Paper

High frequency of Torque Teno virus (TTV) among Egyptian hemodialysis patients

Noha A. Hassuna^{1*}, Medhat Abdel-Fattah², Shrouk Omran², Wael M. Abd Elghany³, Rasha F. Ahmed⁴ and Reham Ali Ibrahim⁵

¹Department of Medical Microbiology and Immunology, Faculty of Medicine, Minia University, Egypt.

²Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Egypt.

³Department of Tropical Medicine, Faculty of Medicine, Minia University, Egypt.

⁴Department of Biochemistry, Faculty of Medicine, Minia University, Egypt.

⁵Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Egypt.

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Very little reports are yet available for the infection rate of Torque Teno virus (TTV) among hemodialysis patients in Upper Egypt. Thus, the aim of this study was to assess the frequency and the possible genotypes of TTV in chronic renal failure patients undergoing dialysis. This cross-sectional study was carried out between August 2016 and February 2017 in three haemodialysis units in Minia, Egypt. Blood samples were collected for serological detection of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) and for detection of TTV. Nested polymerase chain reaction (PCR) was used to detect TTV. Isolates genotypes were identified by sequencing of the N22 region and phylogenetic analysis was also performed. Out of 100 dialysis patients, 76 were TTV positive (52 males and 24 females), with no significant association with gender. TTV was significantly more common among young adults than in older patients. Increased period of haemodialysis posed a high risk for acquiring TTV: Odd Ratio (OR) = 1 and 95% Confidence Interval (CI)= 1.01-1.03. No association was noted between TTV infection and either HCV or HBV infection. Genogroup 1, especially genotype 2 was the most frequently found type in hemodialysis patients. TTV is vastly predominant among Egyptian haemodialysis patients with no significant association with HCV or HBV. Further analyses are recommended to associate the renal failure outcome with the virus load.

Key words: Torque Teno virus, dialysis, phylogenetic biogeography.

INTRODUCTION

Torque Teno virus (TTV) was initially isolated from a Japanese patient having post-transfusional non A-G hepatitis (Nishizawa et al., 1997). The virus is a ubiquitous negative stranded DNA virus, which was classified as a

member of Anelloviridae family (King et al., 2011). The virus is thought to be a hepatotropic virus, which has various modes of transmission (Focosi et al., 2016). According to sequence analysis, TTV is classified into

*Corresponding author. E-mail: nohaanwar@mu.edu.eg.

seven genogroups including many genotypes (Hsiao et al., 2016), of which genotype 1 is the most prevalent (Diniz-Mendes et al., 2004). The virus is globally distributed with variable seropositivity rates according to geographical location (Vasilyev et al., 2009; Spandole et al., 2015b; Amen et al., 2018). TTV commonly infects patients who are exposed to blood transfusion as thalassemic and haemodialysis patients as well as intravenous drug users (Akbari et al., 2018).

The virus has been considered apathogenic and is even considered as a virus flora, however, its importance arises from its ability to mirror the total capacity of innate and acquired immunity (Chen et al., 2013).

Notwithstanding the countless usefulness of haemodialysis for chronic renal failure patients, it has been associated with many complications; it has been attributed as a high-risk environment for blood-borne transmitted infections, which could be through various sources at the haemodialysis units as the equipment, surfaces and personnel (Zuckerman, 2002). In addition to HCV and HBV, TTV is one of the commonly transmitted viruses in patients undergoing haemodialysis, which frequency is widely variable according to virological, demographic and clinical factors (Brajao de Oliveira, 2015). Few studies have reported the frequency of TTV among various Egyptian cohorts with different infection rates (Gad et al., 2002; Hassoba and Khudyakov, 2005), however, these studies are more than 10 years ago and no reports have described TTV frequency in Minia governorate before.

Furthermore, HCV is endemic in Egypt with reported high incidence in Minia region (Hassuna et al., 2015). Thus, the objectives of this work were to evaluate the frequency of TTV viremia in patients undergoing haemodialysis, to evaluate TTV co-infection rates with HBV and/or HCV and to determine the most frequent TTV genotype.

PATIENTS AND METHODS

This is a cross-sectional study carried out in the period from August 2016 to February 2017. Out of 143 patients admitted to three haemodialysis units (Minia University Hospital, Minia General Hospital and Matai General Hospital, Minia governorate); hundred patients, who agreed to participate in this study, were recruited by convenient and consecutive sampling. The study was carried out per the Helsinki declaration (World Medical, 2001) and was approved by the ethical committee of Science Faculty, Beni Suf University.

History taking

Medical records of patients were reviewed to obtain demographic data such as age, sex, underlying diseases; surgical procedures; and haemodialysis history.

Laboratory investigation

A 5 ml blood sample was withdrawn from the patients at the time of

dialysis. Each sample was divided into 2 ml for serum collection for further virological (HCV IgG and HBsAg) and biochemical laboratory tests and 3 mL for detection of TTV in the Microbiology Department, Faculty of Medicine.

Biochemical testing

Alanine amino transferase (ALT) and aspartate transferase levels were assayed on Diatron apparatus (model Pictus B+) according to the manufacturer's kit instructions (BioSystems S.A., Spain). Abnormal values were considered when ≥ 41 IU/L for ALT and ≥ 40 IU/L for AST.

HBV and HCV antibodies detection

Antibodies of HCV (IgG) and HBsAg were detected by mini-VIDAS apparatus (Biomerieux) using corresponding kits: VIDAS anti-HCV and anti HBV (bioMerieux, France) and cut-off values were calculated according to manufacturer's instructions.

Detection of TTV

DNA extraction

DNA was purified from whole blood using the High Pure Viral Nucleic Acid Kit (Roche, Switzerland) according to manufacturer's instructions and DNA was stored at -20°C for further processing.

Nested PCR

Nested PCR protocol was applied for each sample to detect TTV DNA ORF1 (N22) region spanning nucleotides (915-2185) of TTV (Maggi et al., 1999).

The following primers were used for the first PCR round: A5430F (5' CAGACAGAGGAGAAGGCAACATG 3') and A5432F (5'CTACCTCCTGGCATTTCACCA3') and the reactions were carried out in 50 μl volumes which comprised 25 μl of Master Mix (DreamTaq Green Master Mix, Thermo Scientific, USA), 1 μl of each primer, 5 μl template DNA and 18 μl of sterile nuclease free water. Amplification included 35 cycles of 30 s of denaturation at 94°C , 30 s of annealing at 58°C and 45 s of elongation at 72°C , followed by 7 min extension at 72°C . Primers for the second PCR round were: A7861 (5' GGMAAYATGYTRTGGATAGACTGG 3') and NG063 (5' CTGGCATTTCACCATTTCCAAAGTT 3'). Same volumes as aforementioned were used and the cycling conditions were similar to those of the first round of PCR except that the number of cycles was 25. The PCR products were separated on 2% agarose gel and were visualized under ultraviolet (UV) light.

DNA sequencing and phylogenetic analysis

Second round amplicons of the nested PCR were purified and sequenced in both directions using ABI 3500 Genetic Analyzer using BigDye® Terminator V 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Raw DNA sequence reads were further trimmed and contigs were assembled using Vector NTI Express® 1.2.0 (Thermo-Fisher Scientific). Obtained TTV sequences were confirmed by comparison to sequences in the Gene Bank library using BLAST tool (Basic Local Alignment Search Tool; NCBI). Multiple sequence alignment (MSA) and phylogenetic evolutionary analysis for the selected obtained sequences and reference sequences of the main genotypes acquired from the database was carried out deploying Mega7 software (Molecular

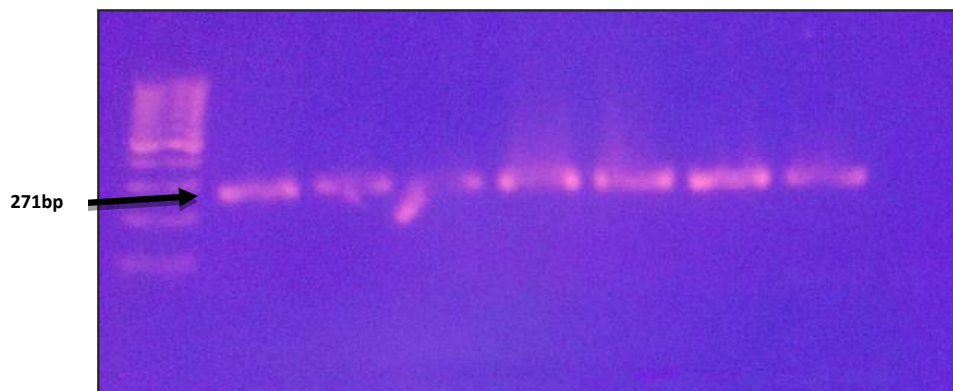


Figure 1. Nested PCR product of the 271bp region of TTV virus.

Table 1. Demographic characteristics and baseline clinical data in TTV-positive and -negative haemodialysis patients.

Variable	Torque Teno Virus (TTV) infection		P-value
	TTV positive (No= 76)	TTV negative (No=24)	
Age	45.7±13.9	52.4±11.8	0.035
Sex	Males (%)	52 (74.3)	0.540
	Females (%)	24 (80)	
Period of haemodialysis (months)	72 (3-216)	48 (5-144)	0.098

Evolutionary Genetics Analysis version 7.0) (Kumar et al., 2016). Evolutionary history was evaluated by the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree deduced from 500 replicates was procured to represent the evolutionary history of the analysed taxa (Felsenstein, 1985). The percentage of replicate trees in which the related taxa grouped together in the bootstrap test (500 replicates) was demonstrated alongside the branches. The Maximum Composite Likelihood method was applied for calculating the evolutionary distances and are in the units of the number of base substitutions per site (Tamura et al., 2004).

Statistical analysis

The statistical analysis was carried out using Prism 6 and SPSS 16.0 for Windows®. Data are presented as mean ± standard deviation (SD), median with range for quantitative variables and number, percentages for categorical variables. Student's t-test was used to compare between two independent means for age and other numeric variable were compared by Mann Whitney test. The Chi-square test was used for categorical variables. P value ≤0.5 was considered statistically significant.

RESULTS

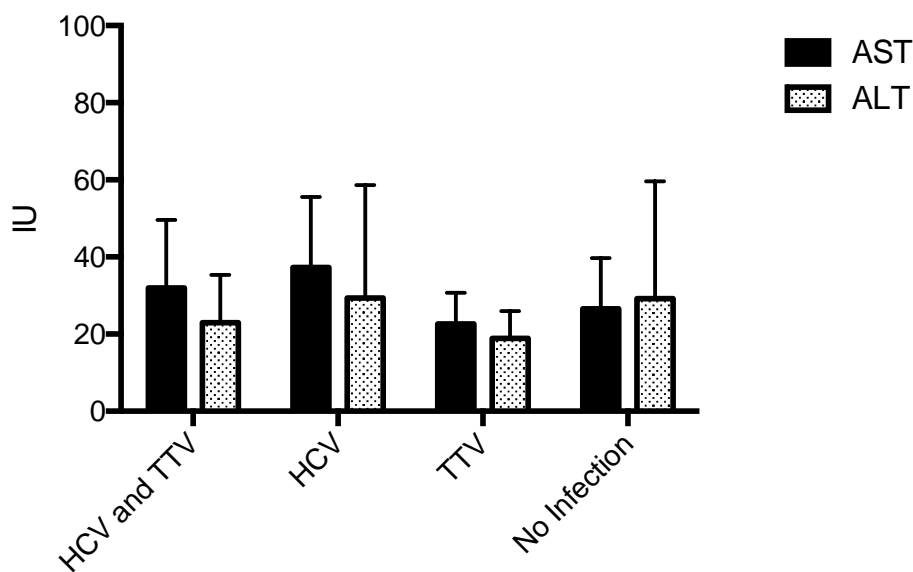
Out of 143 patients attending the three hemodialysis centres, only 100 agreed to participate in the study. Most of the dialysis patients were males (70%) with a mean

age of 47.3 years (standard deviation - SD: 13.7), who spent a mean time of 71.9 months in dialysis with a SD of ±44.8. Regarding the prevalence of TTV infection: 52 males (73.4%) were positive compared to 24 females (80%), which was statistically non-significant with almost comparable or equal prevalence rates ($p=0.540$) (Figure 1). The two groups were significantly associated in relation to age, as TTV was more frequent in relatively younger age (Table 1). Forty-six (60.5%) of the dialysis patients with TTV viremic showed HCV seropositivity, while more than 79% of those without TTV viremia were HCV seropositive. Only two of the TTV positive patients possessed HBsAg, while none of TTV negative had the antigen. Longer period of dialysis was considered as a risk factor for TTV positivity (Table 2). The levels of AST or ALT were not significantly different with HCV alone, TTV alone or with both infections (Figure 2).

Five isolates were randomly selected for DNA sequencing and submitted to GenBank with the following accession numbers: KY750543-KY750547. MEGA 7 software was used to carry out multiple sequence alignments (MSA) for the isolated sequences in addition to reference sequences for TTV acquired from GenBank. Four of the isolates were related to genotype 2 (KY750543-KY750546) and only one isolate was closely related to genotype 1 (Figure 3). The sequence identity

Table 2. Binary logistic regression analysis for detection of variables independently associated with TTV infection in hemodialysis patients.

Variable		Odd Ratio (95% CI)	P-value
Age		0.94 (0.89-0.98)	0.007
Sex	Females	1.0 (reference)	0.2
	Males	1.91 (0.60-6.05)	
HCV and/or HBV	Negative	1.0 (reference)	0.1
	Positive	0.41 (0.12-1.34)	
Duration of hemodialysis (months)		1.01 (1.0-1.03)	0.04

**Figure 2.** AST and ALT levels in haemodialysis patients. Values are the means \pm SD.

for isolates obtained from haemodialysis patients was in the range of 66 to 98%.

Green diamonds represent isolates from this study with accession numbers: KY750543-KY750547. Different genotypes were represented as follows: genotype 1: JaBD28, TA278, TX011; genotype 2: PT3, TS003 and NA004; genotype 3: TKB6; genotype 4: TKM1; genotype 5: THEM 1; genotype 6: TFC3155; genotype 7: THEM2; genotype 8: THEM3.

DISCUSSION

Details concerning the prevalence of TTV in Egypt among different groups are deficient. Up to our knowledge, this is the first multi-centered study evaluating the presence of TTV viremia among haemodialysis patients in Upper Egypt. There is a widely variable geographical distribution

for TTV infection among dialysis patients, which depends on the detection methods, clinical and demographical status of the grouped patients as well as the size of the study.

The frequency of TTV in this study was 76%, which is comparable with Abou-Dounia et al. (2007) whose study was the only one carried on haemodialysis patients in Egypt; nevertheless, it was in Lower-Egypt. Moderately lower frequencies are found in various countries; ranging between 9.3 and 60.9%, which is possibly due to the earlier defined variances between the studied groups specially detection methods (Chan et al., 2000; Kheradpezhohu et al., 2007). A state of endemicity could also explain the high prevalence of TTV in this study as well as some studies carried out in Japan and India (Utsunomiya et al., 1999; Irshad et al., 2010).

Except for age, none of the tested demographic factors (sex or period of dialysis) were associated with the high

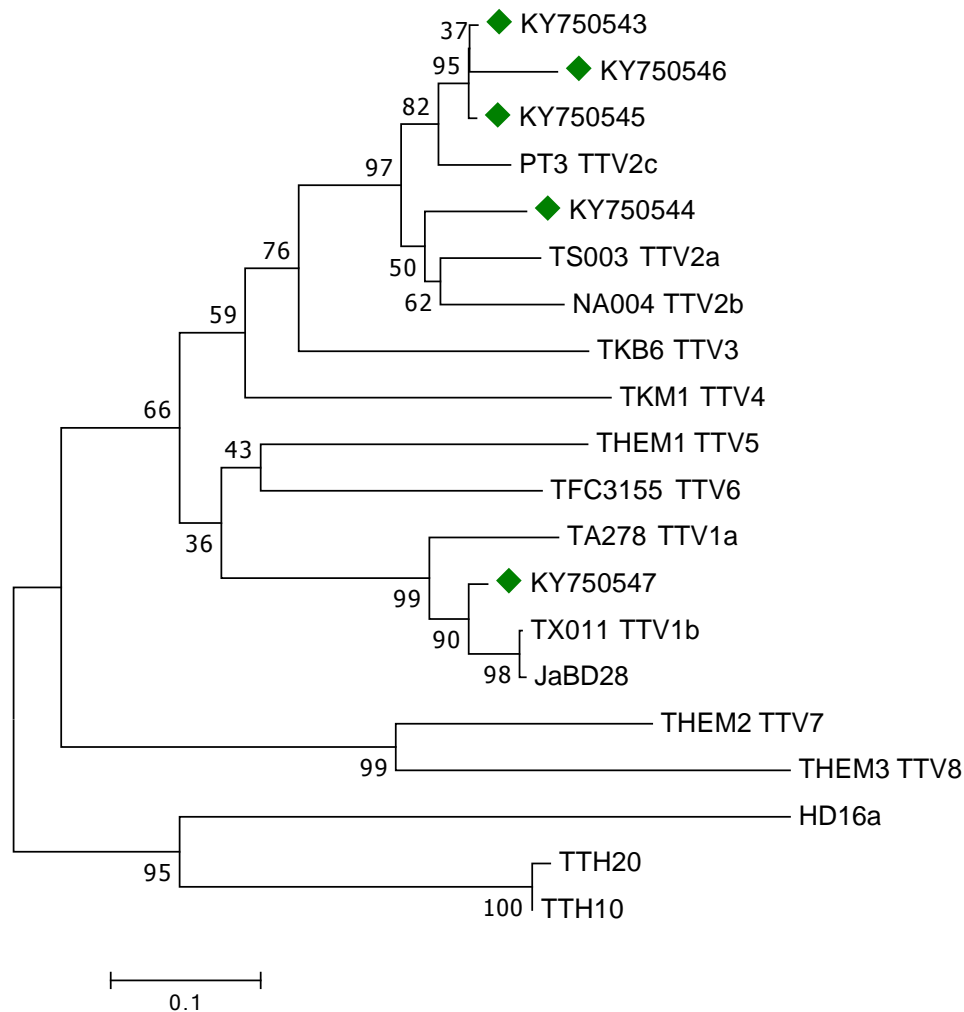


Figure 3. A phylogenetic analysis for the ORF1 region of the TTV. The evolutionary history was inferred using the Neighbour-Joining method using TTV isolates and reference strains.

frequency of TTV, which was in agreement with several studies (Martinez et al., 2000; Kheradpezhohu et al., 2007). An interesting finding was the increased frequency of TTV viremia with younger age, which could be due to possible clearance of the virus with age. Longer period of dialysis poses a risk for acquiring TTV infection, which perhaps could be due to longer exposure duration in addition to the progressive state of immune-deficiency with time in dialysis patients. Furthermore, dialysis techniques also markedly present additional hazard factors for infection in such group of patients (Jalali et al., 2017).

There is a big debate regarding the involvement of TTV in different pathologies especially liver pathology with assumptions that the virus cannot produce the pathology alone (Spandole et al., 2015a); hence, this study evaluated liver enzymes: ALT and AST as biomarkers for liver damage (histological data were not available for this

study) in patients with HCV alone, TTV alone or with both infections. No significant difference was detected in either AST or ALT levels in any of the groups, which concurs with the postulation that TTV is a commensal virus and only certain genotypes and genogroups are associated with liver pathology (Peng et al., 2015; Hazanudin et al., 2019). Further studies are required to evaluate the titre of the virus and its relation with possible renal or hepatic damage as some studies suggested the potential of TTV-induced hepatic damage at certain titres (Simonetta et al., 2017).

Concurrence between TTV and hepatitis viruses has been markedly studied in many reports (Al-Qahtani et al., 2016; Najafimemar et al., 2018). In the current study, seropositivity of HCV IgG was relatively high (65%), while HBsAg was found in only two patients (2%). The high frequency of HCV is similar to an earlier study done in the same region in a similar cohort (Elzorkany and Zahran,

2017). In addition, the HBsAg seropositivity was similar to that found in a study carried out on blood donors in the same area (Hassuna et al., 2015), and is also in agreement with another study carried out by Atwa and Wahed (2017) on transfusion-transmitted infections. Regarding the co-infection status of TTV with HCV and HBV, no significant association between TTV and either HCV or HBV, and despite the fact that the three viruses are parentally transmitted, the lack of concordance could be due to the presence of other non-parenteral routes for TTV transmission.

As mentioned earlier, only certain TTV genotypes are associated with known pathologies: renal pathology (Yokoyama et al., 2002), acute respiratory diseases (Maggi et al., 2003), arthritis (Maggi et al., 2007), laryngeal carcinoma (Hettmann et al., 2016) and post transfusion hepatitis (Tanaka et al., 2000). Accordingly, five TTV isolates were randomly selected and sequenced (not all the isolates were sequenced due to very limited resources) with only four of our isolates closely linked to genotype 2 and only one related to genotype 1. This study is the first to show the prevalent genotypes of TTV among dialysis patients in Egypt with no previous data concerning the prevalent TTV genotypes in Egypt, except for one study carried out by our group on thalassemic patients showing genotype 1 to be the most prevalent (Hassuna et al., 2017).

Limitations to this study included sequencing of few samples for genotyping, which is due to lack of funding and also led to the inability to titrate the virus levels.

Conclusion

The frequency of TTV is relatively high among dialysis patients, especially younger dialysis patients with no significant association with either sex, period of transfusion or HCV/HBV infection. Interestingly, genotype 2 was more frequently found than genotype 1 with high relationship between the viruses. Future measurement of the virus titre could help in evaluating the immunological status of dialysis patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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