
Molecular Detection of Torque Teno Virus (TTV) Infection among Positive HBV Patients in Khartoum State, Sudan

HIND AZHARI¹

Department of Microbiology
Faculty of Medical Laboratory Sciences, Al Neelain University
Khartoum, Sudan

ISAM M. ELKHIDIR

Department of Microbiology and Parasitology
Faculty of Medicine, University of Khartoum
Khartoum, Sudan

ABDEL RAHIM M. EL HUSSEIN

Central Laboratory, Ministry of Science and Technology
Khartoum, Sudan

AHAMED M. ALAMIN

Department of Biochemistry and Molecular Biology
Faculty of Science and Technology, Al Neelain University
Khartoum, Sudan

MOHAMED EL-FATIH

Department of Microbiology
Faculty of Medical Laboratory Sciences, Al Neelain University
Khartoum, Sudan

KHALID A. ENAN

Central Laboratory, Ministry of Science and Technology
Khartoum, Sudan

Abstract:

Background : *Torque Teno Virus (TTV) is a newly discovered non- enveloped, single stranded DNA virus of high genotypic variability, detected frequently in patients with acute or chronic hepatitis of non A-G etiology.*

¹ Corresponding author: sup.222@hotmail.com

Objective: *This study was carried out to look for the presence of TTV among positive HBV patients in Khartoum State, Sudan using polymerase chain reaction (PCR) technique and to study its effect on severity of the disease on these patients.*

Method: *A total of 83 blood samples from HBV positive patients were tested for presence of TTV DNA by polymerase chain reaction (PCR) using primers from untranslated (UTR) region. Also, liver enzyme, alanine aminotransferase (ALT) was measured for each patient.*

Result: *TTV DNA was detected in 24 (28.9 %) of the patients. No detrimental effect of TTV co- infection in viral hepatitis patients was noted.*

Conclusion: *The rate of TTV infection rate among Sudanese HBV patients seems to be lower than that stated in previous reports from other countries. The virus does not seem to aggravate the status of those who are suffering from HBV infection.*

Key words: Torque Teno Virus (TTV), Hepatitis B virus (HBV), polymerase chain reaction (PCR).

Background

A new DNA virus was first isolated from the serum of a patient with post-transfusion non-A-G hepatitis was named first TT virus after the initials of the patient^{1,2} (Torque Teno) but later renamed transfusion transmission virus on the basis of presumed mode of transmission.

TTV is a non-enveloped virus with a single-stranded, circular genomic DNA of approximately 3.9 kb, coated with proteins, and classified as a member of the Circovirus genus in the Anelloviridae family.³ (ICTV, international committee on taxonomy of virus)

In addition to man TTV was also found to infect domestic and wild animals including cattle, chickens, pigs, cats, doge....etc.⁴.

Although the natural mode of TTV transmission is still unclear but there is evidence of its transmission through blood and blood products.^{5,6}

TTV has a worldwide distribution, having been detected in different regions of the world.⁷

Also, the virus has been detected at various body sites other than blood, suggesting that it may be possible to contract TTV infection from environmental sources^{8,9} However, some investigators reported other possible routes of transmission, including vertical,¹⁰sexual,¹¹and oral-fecal¹².

TTV genome is divided into a potentially coding region of 2.8 kb and an untranslated region (UTR) of approximately 1.2 kb. The sequence of TTV genome is very diverse in nature. Up till now 61 different isolates are known worldwide¹³. The cause and mechanism for this great genomic variation are still unknown¹⁴. Presently more than 30 genotypes of TTV have been classified under 5 genogroups¹⁵.

Despite the worldwide distribution and intensive studies of TTV, the association of this virus with any disease is still questionable. In particular, its effect on severity of liver disease, aggravation of hepatic disease condition and progress to complications as cirrhosis and hepatocellular carcinoma in hepatic patients has not yet been clearly defined. Only meager information is available on TTV in Sudan. The present study aimed at investigating TTV prevalence in hepatitis B patient in Khartoum state, Sudan and its possible effects on the disease severity in these patients.

Materials and Methods

Study site:

This study was conducted in Khartoum State, Sudan in patients from dialysis centers (Ebinsina, Alshorta and Alnaw)

and from Saba center (without dialysis) between November 2014 and Feb. 2015. (Table 1)

Sample collection:

Eighty three blood samples were collected from HBV positive patients (68 male and 15 female) (51 from dialysis centers and 32 from the clinic), blood samples were stored at 4 °C till used. Serum samples were obtained and stored at -20 °C.

Data collection:

Informed written consent was sought from all patients. Age, history of blood transfusion, duration of HBV disease, current clinical manifestations and history of jaundice, were recorded for each patient.

Serology:

Serum specimens were tested for HB surface antigen (HBsAg) using commercial enzyme-linked immunosorbent assay (ELISA) kits (Abbot Laboratories, USA) according to manufacturer's instructions and Alanine aminotransferase (ALT) levels were measured using an autoanalyzer (Hitachi 747) (Bicon co., Germany)

Nucleic acid extraction:

Total DNA was extracted from 3ml patients samples using phenol chloroform method ¹⁶. The DNA was dissolved in 50µL tris borate EDTA buffer and used directly for PCR amplification. Extracted DNA was stored at -20 °C till used.

Polymerase Chain Reaction (PCR):

The PCR was performed using primers that are specific for the TTV (5'UTR) conserved regions. The primers used consisted of forward primer T80 (5'GCTACGTCACTAACCACGTG-3') and the reverse primer T935 (5'CTCCGGTGTGTAAACTCACC-3').

The reaction was performed in 20 µL volume of solis Bio dyne master mix (Estonia). The volume included 5µL master mix, 2µL forward primer, 2 µL reverse primer, 2 µL extracted DNA and 14 µL distilled water. The DNA was amplified in thermo cycling condition using PCR machine (Techno Japan) as follow: initial denaturation at 95°C for 10 min, followed by 55 cycles of denaturation at 94°C for 20 sec, annealing at 60°C for 20 sec and extension at 72°C for 30 sec, with final extension at 72°C for 1 min. 10 µL of amplified product was analyzed by gel electrophoresis in 2% agarose stained with 0.15% ethidium bromide and visualized by using UV gel documentation system (INGeNiuse (Germany). The expected size of UTR gene amplicon was 199 pb.

Statistical analysis:

Collected data were analyzed using statistical package for social science (SPSS version 12.0).

A p value of ≤ 0.05 was considered significant.

Results

During the study period, 83 HBV positive patients (68mle and 15 female) were enrolled. Out of these TTV virus was detected in 24 (28.9%) HBV positive samples. (Figure1)

Based on age group, the distribution of patients positive for TTV were (41%) and (40%) in the age groups 20-40 year and 10-20 year old respectively. (Table2).

According to gender, TTV DNA positive in (30%) 21/68 of the male patients and (20%) 3/15 of female patients but with no significant difference between male and female. (Table 2).

According to locality, the highest infection rate (55%) within 9 patients in Bahry,(35%) within 17 patients in Omdorman and (22%) within 57 patients in Khartoum.(Table 2).

Data show no certain symptoms associated with TTV infection. (Tabel3).

No significant ($P \geq 0.05$) effect of TTV infection on the liver was found based on the (ALT) value for all patients. (Table 4).

Figure 1. TTV DNA result (199 bp) on 2% agrose gel. lane1& 2 shows positive samples and lane c-ve show negative control, M: 100bp DNA Marker.

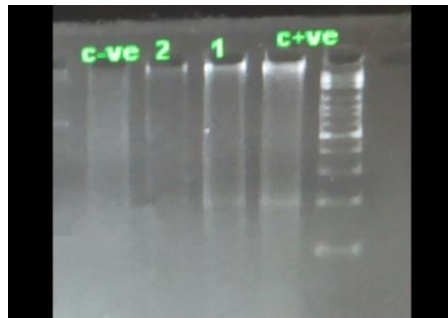


Table 1: frequency distribution of HBV patients tested for TTV From different hospitals

Hospital	Frequency	Percent
Ebnsina-dialysis	35	42.2
Saba clinic	32	38.6
Alshorta-dialysis	10	12.0
Alnaw-dialysis	6	7.2
Total	83	100.0

Table 2: characteristics of TT-virus DNA positive patients

Variable	Number of patients (TTV+ve%)	TTV +ve number (% of total)*	P value
Age (yrs)			Not significant at level ≥ 0.05
10-20	5 (40%)	2(2.4%)	
20-40	39 (41%)	16(19.3%)	
40-60	35 (17%)	6(7.3%)	
60-80	4 (0%)	0(0%)	
Sex			Not significant at level ≥ 0.05
Male	68(30%)	21(25.3%)	
Female	15(20%)	3(3.6%)	

Blood transfusion			
Yes	60(30%)	18(21.7%)	Not significant at level ≥ 0.05
No	23(26%)	6(7.2%)	
Duration of disease (yrs)			Not significant at level ≥ 0.05
Less than 1	29(31%)	9(10.8%)	
1-5	32(31%)	10(12.0%)	
5-10	17(29%)	5(6.0%)	
10-15	4	0(0%)	
Above 15	1	0(0%)	
Locality			Not significant at level ≥ 0.05
Khartoum	57(22%)	13(15.7%)	
Bahry	9(55%)	5(6.0%)	
Om-dorman	17(35%)	6(7.2%)	

*calculated from total patients tested (number=83)

Table 3: clinical manifestation detected in TTV positive & negative patients

Clinical manifestation	TTV+ve (%)	TTV-ve (%)	P value
Headache	1(1.2%)	3(3.6%)	Not significant at level ≥ 0.05
Fever	0(0%)	1(1.2%)	Not significant at level ≥ 0.05
Vomiting	1(1.2%)	2(2.4)	Not significant at level ≥ 0.05
Headache & vomiting	0(0%)	1(1.2%)	Not significant at level ≥ 0.05
Abdominal pain	2(2.4)	1(1.2%)	Not significant at level ≥ 0.05
Jaundice	17(20.5)	34(40.9%)	Not significant at level ≥ 0.05
Nothing	20(24.1%)	51(61.4%)	Not significant at level ≥ 0.05

Table 4: Effect of TTV positivity on the outcome of hepatitis infection as measured by ALT levels

TTV	Number	Mean	SD
Positive	24	49.9174	78.44160
Negative	59	38.9220	79.62919

Not significant at the 0.05 level.

Discussion

TTV is a novel single-stranded DNA virus that is transmitted both parenterally and non-parenterally. Hitherto there has been no clear association with liver disease or any other disease^{17, 18, 19, 20}. Epidemiological studies have shown the virus to be widely distributed in different populations with parenteral risk exposure e.g. hemodialysis patients (19% to 68%), intravenous drug users (19% to 40%), and hemophiliacs (27.4% to 68%). TTV was also detected at a lower prevalence in voluntary blood donors (1.9% to 12%). Moreover, TTV prevalence in apparently healthy population ranging from 7% to 83% was reported in different geographical areas of the world^{21,22}.

In the present study 28.9% of HBV patients is tested positive for TTV.

Studies in other countries showed great variation in positivity of TTV DNA. In Egypt, Omer et al 2006²³ recorded 0% and 27% positivity in HBV and HCV positive patients respectively while 29% and 85% positivity were recorded in blood donors using ORF1 and 5'UTR PCR respectively by other authors^{24,25}. Similar high 88.8% DNA positivity was also reported in Saudi Arabia using 5'UTR PCR²⁶. Thereby comparison, our results indicated moderate positivity.

Our results regarding ALT values corporates the notion that TTV infection has no effect on severity of existing liver diseases²⁷. In our study, the presence or absence of TTV DNA does not seem to affect the clinical course of patients with HBV infection. This indicates that the virus has little influence on the process of inflammation as has been suggested by many authors in different area of the world^{28,29}

Also, in our study, the TTV infection was unrelated to transfusion history as has been indicated by other reports^{30, 31}; but disagree with the earlier observation of Nishizawa et al¹

that TTV is a blood borne infection. Therefore, given that most existing data support the absence of a causal relation between TTV and acute hepatitis, our study also favored the hypothesis that TTV was not responsible as the etiological agent in majority of our cases and probably was innocent bystander.

However, this is contrary to the observation of Desai et al ³²who reported a higher mortality rate amongst TTV patients co-infected with HBV. But we did not find any association between TTV infection and mortality amongst the HBV related patients, and this is also supported by an earlier north Indian study³³. The difference might be attributed to the association of the other risk factors and the possible existence of new, unidentified hepatotropic viruses.

In this study, there was no correlation between age & sex and TTV infection.

These results were in agreement with Abe et al., (1999)³⁴ and Pistello et al., (2001)³⁵, who found no difference in prevalence of TTV infection regarding age or sex.

Conclusion

The present study represents the first report in the prevalence of TTV in HBV patients in Sudan.

TTV does not seem to cause significant liver disease, had no apparent influence on the severity of hepatitis B disease. However, the association between TTV and other persistent agents such HIV, HCV, brucellosis, tuberculosis, toxoplasmosis...etc should be studied.

ACKNOWLEDGMENTS

The authors would like to thank Khartoum hospitals (IbnSina Hospital, El Naw Hospital and. Saba Center), for allowing them to collect the sample from patients, the authors also acknowledge the Department of Microbiology, Faculty of

Medical Laboratory Sciences ElNeelain University, Khartoum, Sudan for funding this research work.

REFERENCES

1. Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology .*Biochem Biophys Res Commun.* 1997; 241: 92-97.
2. Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, et al. Molecular cloning and characterization of a novel DNA virus (TTV) associated with post transfusion hepatitis of unknown etiology. *Hepatology Res.* 1998; 10: 1-16.
3. Mushahwar IK, Erker JC, Muerhoff AS, Leary TP, Simons JN, Birkenmeyer LG, et al. Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. *Proc Natl Acad Sci.USA.* 1999; 96: 3177-3182.
4. Al-moslih M, Perkins H, Hu YW. Genetic relationship of Torque Teno virus(TTV) between humans and camels in United Arab Emirates(UAE). *Journal of Medical Virology.*2007; 79:188–191.
5. Simmonds P, Davidson F, Lycett C, Prescott LE, MacDonald DM, Ellender J, et al. Detection of a novel DNA virus (TTV) in blood donors and blood products. *Lancet.* 1998; 352: 191-5.
6. Kanda y, Chiba S, Tanaka y, Kami M, Saito T, Asai T, et al. TT virus in frequently transfused patients. *Am J Med.* 1999; 106: 116-7.
7. Bostan N, Amen N, Bokhari H: Current and Future Prospects of Torque Teno Virus. Bostan et al., *J Vaccines Vaccin.* 2013;10:1-9.

8. Inami T, Konomi N, Arakawa y, Abe K. High prevalence of TT virus DNA in human saliva and semen. *J Clin Microbiol.* 2000; 38: 2407-8.
9. Osiowy C, Sauder C. Detection of TTV in human hair and skin. *Hepato Res.* 2000; 16: 155-62.
10. Bagaglio S, Sitia G, Prati D, Cella D, Hasson H, Novati R, et al. Mother-to-child transmission of TT virus: sequence analysis of non-coding region of TT virus in infected mother-infant pairs. *Arch Virol.* 2002; 147: 803-812.
11. Krekulova L, Rehak V, Killoran P, Madrigal N, Riley LW. Genotypic distribution of TT virus (TTV) in a Czech population: evidence for sexual transmission of the virus. *J Clin Virol.* 2001; 23: 31-41.
12. Ross RS, Viazov S, Runde V, Schaefer UW, Roggendorf M. Detection of TT virus DNA in specimens other than blood. *J Clin Virol.* 1999; 13: 181-184.
13. Biagini P .Human circoviruses. *Vet Microbiol.* 2004; 98: 95-101.
14. Jelcic I, Hotz-Wagenblatt A, Hunziker A, ZurHausen H, de Villiers EM. Isolation of multiple TT virus genotypes from spleen biopsy tissue from a Hodgkin's disease patient: genome reorganization and diversity in the hypervariable region. *J Virol.* 2004; 78: 7498-7507.
15. Haramoto E, Katayama H, Ohgaki S . Quantification and genotyping of torque teno virus at a waste water treatment plant in Japan. *Appl Environ Microbiol.* 2008; 74: 7434-7436.
16. Agency for Toxic Substances and Disease Registry .Toxicological Profile for Phenol. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2008.
17. Viazov S, Ross RS, Varenholz C, Lange R, Holt-mann M, Niel C, et al. Lack of evidence for an association between TT infection and severe liver disease. *J Clin Virol.* 1998; 11: 183-187.

18. Kanda T, Yokosuka O, Ikeuchi T, Seta T, Kawai S, Imazeki F, et al. The role of TT virus infection in acute viral hepatitis. *Hepatology*. 1999; 29: 1905-1908.
19. Toniutto P, Fabris C, Falletti E, Lombardelli T, Gasparini V, Barillari G, et al. Evidence against a direct role played by transfusion transmitted virus infection in causing hepatic or hematologic manifestations. *Blood*. 1999; 93: 2426-2427.
20. Suzuki F, Kazuaki C, Tsubota A, Akuta N, Someya T, Kobayashi M, et al. Pathogenic significance and organic virus levels in patients infected with TT virus. *Intervirology*. 2001; 44: 291-297.
21. Campo N, Brizzolara R, Sinelli N, Torre F, Russo R, Deferrari G and Picciotto A .TT-virus infection in hemodialysis patients. *Nephrol Dial Transplant*. 2000; 15: 1823-6.
22. Gallian P, Berland Y, Olmer M, Raccah D, De Micco P, et al. TT-virus infection in French hemodialysis patients: study of prevalence and risk factors. *J Clin Microbiol*. 1999; 37: 2538-42.
23. Omar M, Fam N, El-Din SS, Romeih M, Mohamed H, et al. Torque Teno Virus (TTV) Infection in Egyptian Patients with Chronic Liver Disease and Hepatocellular Carcinoma. *Nature and science*. 2006; 4(2):42-50.
24. Abe K, Inami T, Asano K, Miyoshi C, Masaki N, Hayashi S, et al. TT Virus infection is widespread in the general populations from different geographic regions. *J Clin Microbiol*. 1999; 37: 2703-2705.33.
25. Gad A, Tanaka E, Orii K, Kafumi T, Serwah A, El-Sherif A, et al .Clinical significance of TT virus infection in patients with chronic liver disease and volunteer blood donors in Egypt. *J Med Virol*. 2000; 60: 177-181.
26. Maha A, Al-Mozaini, Mohammed N. Al-Ahdal, George Kessie, Damian M, et al. Molecular epidemiology and genotyping of TT virus isolated from Saudi blood donors and hepatitis patients. *Original Article*. 2010; 6(6):444-449.

27. Kao JH, Chen W, Chen PJ, Lai MY, Chen DS. TT virus infection in patients with chronic hepatitis B or C: influence on clinical, histological and virological features. *J Med Virol.* 2000; 60: 387-92.
28. Zhong S, Yeo W, Lin CK, Lin XR, Tang MW, et al. Quantitative and genotypic analysis of TT virus infection in Chinese blood donors. *Transfusion.* 2001; 41: 1001-1007.
29. Hsieh SY, Wu YH, Ho YP, Tsao KC, Yeh CT, et al. High prevalence of TT virus infection in healthy children and adults and in patients with liver disease in Taiwan. *J Clin Microbiol.* 1999; 37: 1829-1831.
30. Utsunomiya S, Yoshioka K, Wakita T, Seno H, Takagi K, Ishigami M, et al. TT virus infection in hemodialysis patients. *Am. J Gastroenterol.* 1999; 94: 3567-70.
31. Ikeuchi T, Okuda K, Yokosuka O, Kanda T, Kobayashi S, Murata M, Hayashi H, et al. Superinfection of TT virus and hepatitis C virus among chronic haemodialysis patients. *J Gastroenterol Hepatol.* 1999; 14: 796-800.
32. Desai MM, Pal RB, Banker DD. Molecular epidemiology and clinical implications of TT virus (TTV) infection in Indian subjects. *J Clin Gastroenterol.* 2005; 39: 422-9.
33. Irshad M, Sharma y, Dhar I, Singh J, Joshi yK. Transfusion transmitted virus in association with hepatitis A-E viral infections in various forms of liver diseases in India. *World.J Gastroenterol.* 2006; 12: 2432-6.
34. Abe K, Inami T, Asano K . TT virus infection is wide spread in the general populations from different geographic regions. *J Clin Microbiol.* 1999; 37: 2703 - 2709.
35. Pistello M, Morrica A, Maggi F. TTV levels in the plasma of infected individuals with different hepatic and extra-hepatic pathology. *J Med Virol.* 2001; 63:189-195.