Microbiology Research Journal International

23(2): 1-6, 2018; Article no.MRJI.38077
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Molecular Detection of Torque Teno Virus among HIV Seropositive Patients in Khartoum, Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAE and ARMEH managed the analyses of the study. Author IME managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/38077
Editor(s):
(1) Lachhman Das Singla, Professor, Department of Veterinary Parasitology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, India.
Reviewers:
(1) Samander Kaushik, MD University Rohtak, India.
(2) Adelabu Olusesan Adeyemi, University of Fort Hare, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/23874

Original Research Article

Received 10th November 2017
Accepted 24th January 2018
Published 28th March 2018

ABSTRACT

Background: Torque Teno Virus (TTV) is a newly discovered non- enveloped, single-stranded DNA virus of high genotypic variability, frequently detected in patients with acute or chronic hepatitis with non A-G etiology.
Objective: This study was carried out to look for the presence of TTV among HIV seropositive patients in Khartoum State, Sudan using polymerase chain reaction (PCR) technique.
Methods: A total of 44 blood samples from HIV positive patients were tested for the presence of TTV DNA by polymer as chain reaction (PCR) using primers from UN translated (UTR) region.
Results: During the study period, 44 HIV positive patients (16male and 28female) were enrolled. Out of these TTV viri was detected in 10(22.7%) HIV positive samples.
Conclusion: The rate of TTV infection among Sudanese HIV patients was (22 %(10/44).

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Keywords: Torque Teno virus (TTV); human immunodeficiency virus (HIV); polymerase chain reaction (PCR); UN translated (UTR).

1. INTRODUCTION

Torque Teno virus (TTV) was first discovered in 1997 in Japanese patients with non-A-G transfusion-acquired hepatitis [1]. TTV is a small, non-enveloped virus with a single-stranded, circular DNA genome of negative polarity, 3.4-3.9 Kb in length, containing two bigger (ORF1 and ORF2) and several smaller open reading frames [2]. TTV is currently classified as Circoviridae family [2]. The family Circoviridae includes two genera, Circovirus and Cyclovirus [3]. Members of the genus Circovirus have only been identified in vertebrates, whereas members of the genus Cyclovirus have been identified in both vertebrates and invertebrates [4]. The type species of the genus Circovirus is Porcine circovirus 1 and the type species for the genus Cyclovirus is Human-associated cyclovirus 8. The species demarcation threshold for viruses of the family Circoviridae is 80% genome-wide nucleotide sequence identity. Members of the genus Circovirus have the ori on the same strand as the rep ORF, whereas members of the genus Cyclovirus have the putative ori on the same strand as the cp ORF [4]. Circovirus genomes are characterised by two intergenic regions between the significant ORFs; however, the intergenic region between the 3¢ ends of the major ORFs in cyclovirus genomes is either absent or consistently smaller [5]. Besides, introns have been identified within the ORFs of several cyclovirus genomes, while none have been observed for members of the genus Circovirus.

Members of the family have two significant ORFs encoding replication-associated (Rep) and capsid (Cp) proteins, as well as a conserved nonanucleotide motif marking the origin of replication. The nonanucleotide motif sequence is depicted through sequence probability logos generated in Weblogo 3. The rep gene of human-associated cyclovirus 8, a representative of the Cyclovirus type species, is interrupted by an intron. The ori is characterized by a conserved nonanucleotide motif [(T/n) A (G/t) TATTAC] at the apex of a stem-loop structure located between the 5¢ ends of Rep- and Cp encoding ORFs [4,6]. In characterised members of the genus Circovirus, the Rep protein is thought to initiate replication through the rolling circle replication (RCR) mechanism by nicking the virion-sense strand between positions 7 and 8 of the nonanucleotide motif [7]. RCR involves the production of a dsDNA replicative form by host DNA polymerases and the generation of viral ssDNA from the replicative form template. Both circovirus and cyclovirus Rep proteins contain conserved domains that are important for RCR. Putative Rep-binding domains characterised by iterative sequences near the ori have been identified for members of both genera [8,9].

Despite being a DNA virus, TTV exhibits an extensive sequence divergence. At least 16 genotypes with evolutionary distance >0.30 have been described so far [10]. TTV is a ubiquitous virus revealed in more than 50% of the general human population throughout the world [11,12] and nearly 90% of ponged communities [13]. Co-infection of single individuals with TTV isolates belonging to one or several phylogenetic groups frequently occurs [14]. TTV was first characterised as a blood-borne virus and thus referred to transfusion-transmitted (TT) group of infections [1]. However, recent studies suggested the existence of other ways of transmission including parenteral [10], sexual [15,16], mother-to-child [17,18] and others [19,20]. TTV has also been suggested to be a causative agent of several diseases such as acute respiratory diseases [21], liver diseases [22,12], AIDS [23] and cancer [24], but without any convincing support. One of current hypothesis suggests a crucial role of TTV in development of autoimmune reactions [25]. Despite years of investigation, the TTV distribution in humans is still a subject of discussion. Possibly, this is because of the variability of TTV genotypes and the inability to design a single set of PCR primers, corresponding to the vast majority viral genotypes [26]. Little is known about the distribution of TTV in HIV seropositive patients in Sudan. In this study, we investigated the existence of TTV viral DNA in the blood of 44 Sudanese HIV seropositive patients.

2. MATERIALS AND METHODOLOGY

2.1 Study Design

This is a Cross sectional study carried out in Khartoum state's hospitals.

2.2 Clinical Samples

This study was conducted in three Khartoum Hospitals (Basher Hospital, Khartoum Hospital

and Omdurman Hospital) during period March to October 2017. All participating patients were given a written informed consent.

Blood samples from 44 patients with HIV (16 males and 28 females) were collected in EDTA tubes and centrifuged at 3000 RPM for 5 minutes. Obtained plasma used for rapid Enzyme Linked Immunosorbent assay (ELISA) and DNA extraction for polymerase chain reaction PCR. The viral DNA was finally eluted in 60µl of elution buffer and stored at -20°C. All the patient samples were tested by ELISA to confirm seropositivity of HIV.

2.3 Serology

Commercial ELISA kits (Chemo BioScience, INC, San Francisco, USA) were used to Confirm seropositivity for HIV according to the procedure described by the manufacturer.

2.4 DNA Extraction

Total DNA was extracted from 200 µl patient's serum using DNA extraction kit (analytikjena, Germany). DNA was finally eluted in 60 µl of elution buffer and stored at -20°C.

2.5 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 primerT80 (5'GCTACGTCACTAACCACGTG-3') and the reverse primer T935 (5'CTCCGGTGTGTAAACTCACC-3'). The reaction was performed in 20 µL volume of soils Bio dyne master mix (Estonia). The volume included 5µL master mix, 2µL forward primer (10 pg), 2 µL reverse primer (10 pg), 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 95ºC for 10 min, followed by 55 cycles of denaturation at 94ºC for 20 sec, annealing at 60ºC for 20 sec and extension at 72ºC for 30 sec, with final extension at 72ºC 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 bp.

2.6 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.

3. RESULTS

Out of 44 HIV seropositive patient 10 (22.7%) were found positive for TTV DNA by polymerase chain reaction (Fig. 1).

Based on age group, the distribution of patients positive for TTV were (60%) and (40%) in the age groups 20-40 year and 40-60 year old respectively (Table 1) (p value 0.083).

According to gender, TTV was positive in (37.5%, 6/16) of the male patients and (14.2%, 4/28) of female patients but with no significant difference between male and female (P value 0.512) (Table 1).

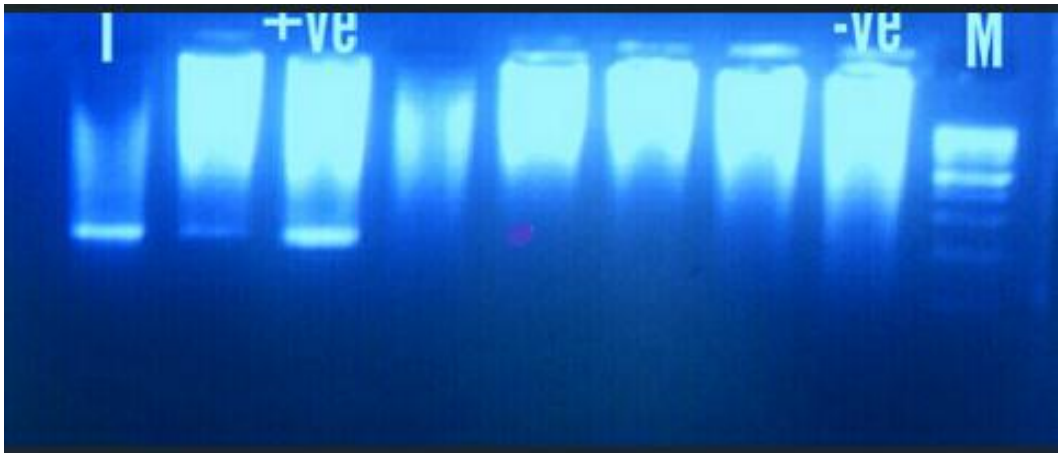

Fig. 1. TTV DNA result (199 bp) on 2% agarose gel. Lane 1 show positive sample and lane +ve, -ve show positive and negative control respectively, lane M: 100bp DNA Marker

Table 1. Frequency distribution of TTV according to the age and gender

Variable	Number of patients (TTV+ve%)	TTV +ve number (% out of total)*	P value
Age (yrs)			
20-40 n 20	6(60%)	6(13.6%)	Not significant at level
40-60 n 24	4(40%)	4(9%)	≥0.05
Sex			
Male n 16	6(60)	6(13.6%)	Not significant at level
Female n 28	4(40)	4(9%)	≥0.05

Table 1 shows the frequency distribution of TTV according to the age and gender with no significant differences.

4. DISCUSSION

TTV is a novel single-stranded DNA virus that is transmitted both parentally and non-parentally. Hitherto there has been no clear association with liver disease or any other disease [23,24,25,26]. 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. [27,28]. In another study TTV DNA, detected by PCR with UTR primers was present in 185 of 226 (81.8%) healthy individuals and in Italy [29].

In an earlier study in Sudan by Azhari et al. [30] out of 83 (28.9 %) of HBV patients were positive for TTV using PCR .In the present study 10/44 (22.7%). of HIV patients tested positive for TTV using the same technique. These results are higher than those (15%, 8/52) reported by Geers et al. [31] In Italy.

However Debiaggi et al. [29] reporeted that TTV DNA was found in 229 of 238 (96.5%) HIV-1-seropositive patient's in Italy. Thereby confirming the findings previous studies of a deep and wide penetration of TTV (with various genotypes) into the community. Furthermore, TTV DNA detectable by PCR with N22 primers was present with a similar prevalence in populations with different risks factors including multiple-transfused patients and bone marrow transplant recipients [32,33,34].

5. CONCLUSION

In the present study 22.7% of our study population was found positive for TTV using PCR, and no significant differences according to age and sex were discernable.

Finally, our study represents the report on TTV infection in HIV patients in Sudan.

ETHICAL REVIEW

The study was approved by the Ethical Review Committee (ERC) of Alneelain University, the Ministry of Higher Education & Scientific Research, Khartoum State, Sudan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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