Citation: Nasr Al Deen M. Bush, Salah Al Deen G. Al Zaki, Salwa H. Mohammed, Huda A. Al Tyeb, Abbas Bakhit, Mustafa Al Tigani. Molecular Detection of Torque Teno Virus among Blood Donors attending Nyala Teaching Hospital (Sudan). African Journal of Medical Sciences, 2018, 3 (8) ajmsc.info

Molecular Detection of Torque Teno Virus among Blood Donors attending Nyala Teaching Hospital (Sudan)

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Abstract

Background: 80% of hepatitis cases are caused by five types of hepatitis viruses (A-E) known as hepatotropic viruses, The remaining 20% cases and 10% transfusion associated hepatitis cases are negative for viral hepatitis infection, suggesting the existence of other hepatitis agents. Transfusion transmitted virus or torque teno virus (TTV) was first reported in a Japanese patient in 1997 by T. Nishizawa. The virus is extremely common, even in healthy individuals, as much as 100% prevalent in some countries, and in approximately 10% of blood donors in the United Kingdom and USA. Although it does not appear to cause symptoms of hepatitis on its own, it is often found in patients with liver disease and blood of people with hepatic pathologies of unknown etiology. For the most part, TTV infection is believed to be asymptomatic.

Objective: To perform molecular detection of Torque Teno Virus among blood donors attending Nyala Teaching Hospital (Sudan)

Materials and methods: TTV DNA genome was determined using polymerase chain reaction (PCR) in serum samples from 150 healthy blood donors attending Nyala Teaching Hospital.

Results: TTV was detected in 17 (11.3%) out of 150 blood donors.

Conclusion: The study indicated a moderate seropositivity of torque teno virus among the blood donors investigated.

Key words: Torque teno virus, PCR, Blood donors, Nyala Teaching Hospital.

Introduction

The Torque teno virus (TTV) was first detected in 1997 in the blood of Japanese patients with post-transfusion hepatitis. The virus was also detected in the liver and blood of people with hepatic pathologies of unknown etiology. The association between TTV and liver diseases is still controversial and several studies have been undertaken to identify infection sources.

Epidemiological studies had evidenced the prevalence of TTV in other pathological conditions, such as autoimmune diseases, respiratory conditions and cancer. However, information is still lacking on TTV infection and on the development of pathologies, as well as the change in the course of a particular disease[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. TTV is currently classified as one of the *Circoviridae* family. Despite being a DNA virus, TTV demonstrates an extremely wide sequence divergence. At least 16 genotypes with evolutionary distance >0.30 had been described so far. TTV is an ubiquitous virus revealed in more than 50% of the general human population throughout the world, and nearly 90% of pronged populations. Co-infection of single individuals with TTV isolates belonging to one or several phylogenetic groups can occur frequently[2].

TTV was first characterized as a blood-borne virus and thus referred to as transfusion-transmitted (TT) group of viruses. Recent studies suggested the existence of other ways of transmission including parenteral, sexual, Mother-to-child, and others. TTV has been suggested to be a causative agent of several diseases such as acute respiratory diseases, liver diseases, AIDS, and cancer, without any convincing support. One of current hypothesis suggests a key role of TTV in development of autoimmune reactions. But until now no confirmed disease associations, and to date, there are no reliable commercial serological assays that can be used for large-scale screening. After the discovery of TTV, five other novel Circoviruses were reported. These include: SANBAN virus, TTV-like mini virus (TLMV), SEN virus (SENV), Sentinel virus (SNTV) and YONBAN virus. Clear disease associations for these agents are obscure[3].

Materials and methods

This was a descriptive-cross sectional study conducted at Nyala Teaching Hospital during the period from October 2017 to March 2018. Ethical clearance was obtained from the Research Ethical Committee of Al Neelain University. A verbal consent was obtained from all blood donors investigated. The software used for the data analysis was the Statistical Package for Social Sciences (SPSS). For categorical variables, proportions were compared by the chi-square test as appropriate. The means and medians of the continuous variables were compared by Student's *t* test depending on the sample distribution. Frequencies, percentages, and graphs were used for presentation of the data.

Confidentiality of information regarding patients investigated was maintained. Permission to collect the specimens was obtained from Nyala Teaching Hospital.

Sampling was a non- probability purposive sampling type, and sample strategy was convenience where the study population was selected on the basis of accessibility. Clinical and demographical data was collected using a direct interviewing questionnaire.

150 blood samples were collected from healthy blood donors' volunteers meeting the standard eligibility criteria for donation (i.e. negative tests for hepatitis B surface antigen and for antibodies of HCV and HIV.

2 ml blood was collected from each donor, centrifuged at 3500 rpm at room temperature. Sera were separated and transferred to fresh tubes. The serum samples were kept at -20°C until

molecular investigation is performed.
Molecular investigation: Samples were tested for TTV genome by the polymerase chain reaction (PCR). 500 µl Serum were mixed with 250 µl of 0.5% SDS. 20 µl of 10 mg/ml proteinase K solution was added and incubated at 56°C for 2 hours, then the samples were left at 95°C for 10 minutes to deactivate proteinase K. Protein was precipitated with two phenol: chloroform: isoamyl alcohol (25:24:1) solution and followed by chloroform treatment. The cold absolute ethanol (100%) and (70%) ethanol were used for DNA precipitation. The precipitate was dissolved in 250 µl of deionized water and preserved at -20°C[4].
Primers used were specific for the TTV N22 region and (5'UTR) conserved regions. For single step PCR, the primers consisted of:
Forward primer: T80 (5'GCTACGTCACTAACCACGTG-3')
Reverse primer: T935 (5'CTCCGGTGTGTAAACTCACC-3').
The reaction was performed in 20 µL volumes of Solis Bio Dyne master mix (Estonia). The mixture used was 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 PCR machine (Techno Japan) as follow:
Initial denaturation at 95ºC for 10 min, following 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 pone min. Then 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 of INGeNiuse (Germany). The expected size of UTR gene amplicon was 199 bp.

Gel electrophoresis was performed by diluting Tris-borate-EDTA buffer (10 ×) 10 times by adding 100 ml of Tris-borate-EDTA buffer (10 ×) with 900 ml distilled water, and one gram of agarose powder and dissolved in 50 ml of (1 ×) Tris-borate EDTA buffer to a final concentration of 2% agarose buffer.
The last solution was boiled in the microwave, then cooled at room temperature and 5 µl ethidium bromide were added to stain the gel. The solution was poured into the gel tray and the comb was placed to make a well on which the PCR products were loaded.
After amplification, the product was run on 2% agarose gel electrophoresis in Genei TM machine (Bangalore, India). The PCR products, positive and negative control, and 10µl were loaded in each well according to the prepared master chart. 100 base pair ladder (Fermentas, USA) was used for comparison (Fig. 1).

Results

From the150 blood donors investigated, 59 (39%) were aged 16-26 years, 73 (49%) were aged 27-37 years, and 18 (12%) were aged 38-44 years. The frequency rate of TTV seropositivity was 47.4% in the age group 16-26 years, 52.6% in the age group 27-37 years, and none in the age group 38-44 years (Fig. 2).
No association was found in the frequency rates of TTV infection between the seropositivity of the age group 16-26 and the age group 28-38 years (Chi-square =0.053, and p = 0.819).

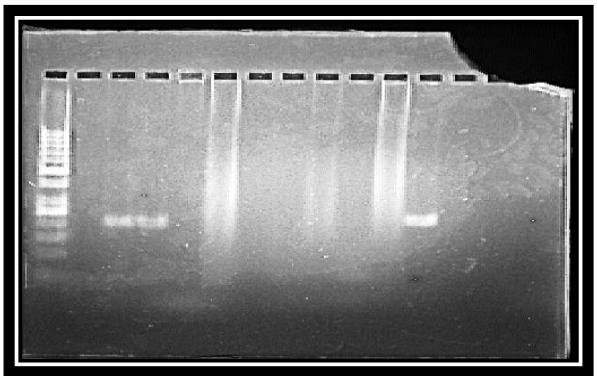

Fig. (1): PCR detection of TTV

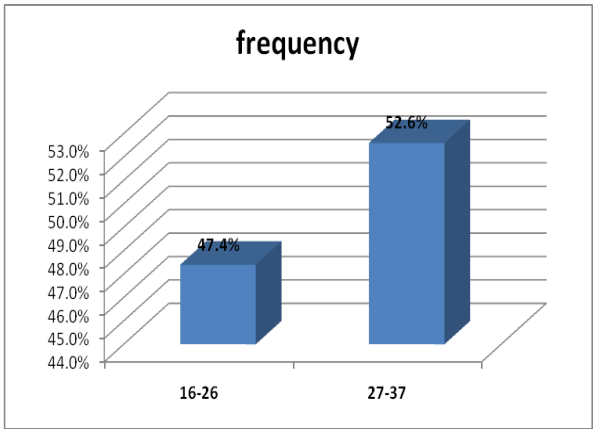

Fig. (2): Frequency rate of TTV seropositivity in age group 16-26 years and 27-37 years

In this study, 17 cases (11.3%) were found TTV positive. The majority of positive blood donors were blood group O positive; and the least positive were blood group O negative (Table 1). No significant association was found in the frequency rates of TTV infection between the seropositivity and blood groups of the cases investigated.

Table (1): Distribution of positive TTV according to blood groups

Blood group	Frequency rate	%
A +ve	4	23.6%
B +ve	2	11.8%
O -ve	1	5.8%
O +ve	10	58.8%

Discussion

The present study indicated that TTV is a relatively common viral infection (11.3%) among healthy blood donors attending the Blood Bank Center at Nyala Teaching Hospital. The tendency of prevalence towards a younger age for TTV–positive was between the ages 16-38 years versus TTV–negative blood donors provided some insight to the routes of infection, i.e. an association with young age would be highlighted in the future.

This study did not genotype TTV in the Sudanese community. Absence of such a technique created some limitation of our study.

In various countries, researchers reported prevalence rates similar to what was documented in our study. Jie and his colleagues in China (2001) reported a 14.8% positivity of TTV DNA[5]. Also, Juhng and his co-workers[6] in Korea, detected an 8.2% sero-prevalence rate among blood donors. In an Iranian report the frequency rate was 27.8% among blood donors[7].

In Qaluobia governorate (Egypt), Hafez and his co-authors (2007) found a sero-prevalence rate of 36.7% among blood donors[8].

Furthermore, a high finding (83%) of positivity was reported in Khartoum State (Sudan) by El Hadi using 5'UTR PCR[9].

Conclusion: The study indicated a moderate seropositivity of torque teno virus among the blood donors investigated.

Acknowledgments: We thank all staff of Nyala Blood Bank Center for their technical assistance and their effort in collecting the blood samples. Also, our gratitude goes to All staff of the Department of Epidemiology and Tropical Medicine for performing the molecular analysis of this study.

Conflict of interests: The authors did not declare any conflict of interest.

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