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Research Article

PREVALENCE OF TRANSFUSION TRANSMITTED VIRUS (TTV) AMONG HEPATOCELLULAR CARCINOMA PATIENTS IN SUDAN

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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 types. **Objectives:** This study was carried out to look for the presence of TTV among positive hepatocellular carcinoma (HCC) patients in Khartoum State, Sudan using polymerase chain patients. (PCR) technique and to evaluateits effect on severity of the disease in these patients. **Patients and Methods:** The study was conducted in 45 patients with HCC recruited at Aliaa specialist hospital in Khartoum state, The presence of TTVDNA was assessed by polymerase chain reaction with TTV-specific primers. **Results:** DNA of TTV was detected in 4 out of 45 (8.9%) patients with HCC, no detrimental effect of TTV co- infection in viral hepatitis patients was noted. **Conclusion:** The rate of TTV infection rate among Sudanese HCC patients seem 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 HCC.

Keywords: Torque Teno Virus (TTV), Hepatocellular carcinoma (HCC), Polymerase chain reaction (PCR).

INTRODUCTION

In 1997 Torque Teno virus (TTV) was first described in a Japanese patient with non-A-G transfusion-transmitted hepatitis of unknown etiology (1-5). Torque Teno Virus (TTV) is a circular ssDNA virus (1). TTV was proposed as a member of a new family named "Circoviridae" and its genus is "Anelloviridae" (2). TTV is unique in many ways, as it was the first single stranded circular DNA virus discovered that infects humans (3).Visualization of TTV derived from clinical samples of infected individuals showed a non-enveloped virus with single- stranded circular DNA genome of negative polarity, 3.4-3.9 Kb in length, and several open reading frames (4, 5). The genomic length varies depending on genotype, and measures 3,853 bases in the prototype (6). Human TTV is also known as an orphan virus (viruses that are not associated with any disease but may have pathogenic effect) (7). TTV has been found in many body fluids including saliva, milk, tears and feces. Parenteral, fecal-oral, mother to fetus and sexual routes have been suggested for TTV transmission (8). Although TTV can be transmitted through blood or blood products, these routes of transmission do not explain the high prevalence of TTV in blood recipients (9). TTV has been suggested to be a causative agent of several diseases such as acute respiratory diseases [6], liver diseases [11,12], AIDS [22] and cancer [23], albeit, without any convincing support. One current hypothesis suggests a key role of TTV in development of autoimmune reactions [24]. But until now no confirmed disease associations have been discerned, and to date, there are no reliable commercial serological assays that canbe used for large-scale screening. Several clinical findings together with detected TTV DNA in 47 % of patients with fulminant hepatitis and in 46 % of patients with chronic liver disease of unknown etiology support the proposal that TTV might deserve partly be a possible agent for acute and chronic liver diseases of unknown etiology (10). Although it was first detected in patients infected by transfusion, TTV is distributed in more than 50 % of normal human population throughout the world suggesting many ways of virus transmission (11). In relation to hepatitis, some noteworthy observations have surfaced. In one study the histological grade score was higher in case of Hepatitis C Virus (HCV)-TTV co-infection as compared to infection with HCV only (12). There have been claims that TTV viral load is a significant factor for hepatocellular carcinoma (HCC) in hepatitis C patients, irrespective of known risk factors (13). In addition to man, TV was also found to infect domestic and wild animals including cattle, chickens, pigs, cats, doge…. etc. Previous studies In Sudan showed that *TTV DNA was detected in 24 (28.9 %) of HBV patients with no detrimental effect (14), in 10 (22%) out of 44 HIV patients,* in 3(5 %) out of 60 blood samples from people who have contact with domestic chickens. According to the paucity of data about the prevalence of TTV in Sudan, this study was conducted to generate preliminary data on infection rate of TTV in HCC patients in Khartoum State, Sudan.

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MATERIALS AND METHODS

This study was carried out on HCC patients admitted to Aliaa specialized Hospital, Khartoum. After explaining the purpose of the study, and after obtaining verbal approval, demographic data were collected from 45 volunteers who agreed to participate in the study by interviewing questionnaire. Collected data included gender, age, and health status. The obtained data were saved for statistical analysis. A total of 45 blood samples (5mleach) were obtained from the participating HCC patients. Blood samples were centrifuged at 5000 rpm for 5 minutes then serum was collected and stored at -20 ˚C till

tested. TTV DNA was extracted from serum using Viral Gene-spin™ Kit RNA / DNA Isolation Kit (Intron Biotechnology Company, Korea) following the manufacturer's procedure, DNA pellets were then collected and stored in 4 C° for PCR testing. Commercial ELISA kits (Chemo Bioscience, NC, San Francisco, USA) were used to Confirm seropositivity for HCV according to the procedure described by the manufacturer.

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'CTCCGGTGTGTA AACTCACC-3') (15). The reaction was performed in 20µl volume using Maxime PCR Premix (cat.No.25026) beads. The volume included: lyophilized master mix, 2 µL; forward primer, 2 µL reverse primer, 3 µL extracted DNA and 13 µL distilled water. The mixture amplified in thermo- cycling conditions using PCR machine (Techno Japan). Check this part especially volumes. The thermo cycling condition were 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.10µl of the amplified product was subjected to direct analysis by Gel Electrophoresis The expected product (199 bp in size) was visualized by staining with ethidium bromide using UV gel documentation system (15).

Statistical analysis

Collected data were analyzed using statistical package forsocial science (SPSS version 12.0).A p value of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

TTV has been suggested to be a causative agent of several diseases such as acute respiratory diseases, liver diseases, AIDS, development of autoimmune reactions, and cancer albeit, without any convincing evidence (16). Some scientists expressed the view that TTV can be part of the human virama due to its nonpathogenic nature and high prevalence and persistence in the body (17).TTV DNA level in patients undergoing organ transplantation has also been suggested to serve as an endogenous marker of the body's immune status (18). A total of 45 Sudanese HCC subjects were enrolled in this study, including patients diagnosed with positive and negative Hepatitis C virus (HCV) infection. The result showed 75% (3) of males were positive for HCV infection while 25% (1) of female were positive for virus HCV. Twenty two (55%) of the patients were males and 19(45%%) were females. (Figure2) (Figure 3). The result reported high frequency of infection (50%) among age group (70-80) then (25%) among age group (80-90) and (40-50) were positive with HCC infection with no significant difference (Figure 4). In the present study, 4(8.9%) of our HCC patients showed positive TTV DNA in their serum while 41 (91 %) of the them tested negative.(Figure 1), This is much lower than the prevalence noted in Sudan *in HBV patients(28.9%) (17), in HIV (22%) patients (15), but higher than that* (5 %) observed in people who have contact with domestic chickens (19). It is also much lower than the prevalence reported in HCC patients in Japan(92%), Korea(67%), and Egypt (31.8%; 46,7%) {13, 20, 21, 22) and HCV patients in Qatar(84,9%) and UAE nationals (95.7%)*(23,24),It was also noted that coinfection no untoward effects in our cohort of HCC patients which is accordance with many studies worldwide*

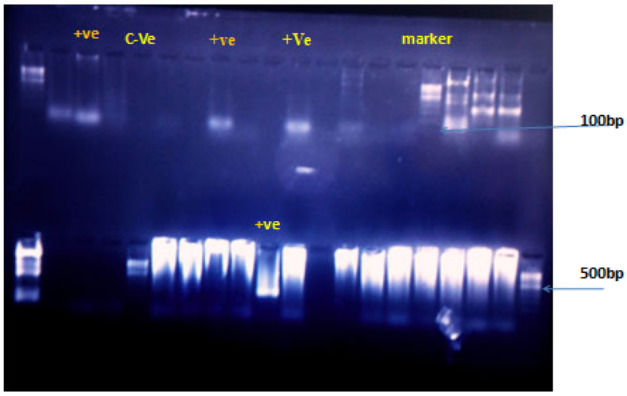

Figure 1. TTV DNA result (199 bp) on 2% agarose gel. lane1& 2 shows positive samples and lane c-ve show negative control, M: 100bp DNA Marker

The result showed a statistically insignificant relationship between patients' gender and infection with TTvirus.*(P.value*=0.394) .(Table1)

Table 1. Association between patients' gender and infection with TT virus

Gender	Total tested	TTV infection		P.value
Male	25	3	12%	0.394
Female	20	1	5.2%	

The result showed a statistically in significant relationship between age group and infection with TTV. *(P.value*=0.66) (Table 2).

Table 2. Association between age group and infection with TT virus

Age group	TTV infection Positive	Negative	P.value
20-30	0	2	
30-40	0	2	
40-50	1	4	0.667
50-60	0	5	
60-70	0	11	
70-80	2	14	
80-90	1	3	

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