Molecular detection of Torqueteno virus in healthy blood donors in Khartoum-state, Sudan

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Abstract:

Torqueteno virus (TTV) is a recently discovered virus with a high DNA prevalence in different populations. Its role in pathogenesis is uncertain. This study was conducted to determine the existence of Torqueteno virus DNA (TTV DNA), and the prevalence of (TTV), among blood donors in Khartoum State, and to generate preliminary rate of infection. The methods used in this present study were conventional PCR diagnostic methods, amplifying the untranslated region (UTR) of (TTV) in serum samples. The consensus primers used are T801 and T935, both primer are able to identify all the genotypes of this virus. 81 Sudanese blood donors, (volunteers) admitted to blood bank Soba Hospital, south of Khartoum were selected for this study. The ages of samples ranged from 18 to 60 years with the mean age of 39 years. Samples were collected throughout the period of October to November 2012. The results which are statistically analyzed show that there was No significant differences in DNA prevalence of (TTV) among blood donors ($P>0.05$) in their serum samples. The present study is one of first attempts to reports directly major infection rate of TTVDNA in blood donors in Khartoum state Sudan, and focus on the molecular diagnosis of TTV DNA. It revealed that TTV was circulating in Sudan among healthy blood donors. The results of present study demonstrated a high prevalence of TTV in blood donors in Khartoum State-Sudan. Further
studies are required to determine prevalence of TTV in other groups and the role of TTV in the pathogenicity of acute and/or chronic liver disease.

**Key words:** Torqueteno virus (TTV), blood donors, untranslated region (UTR), TTV DNA, PCR.

**Introduction:**

Torgue teno virus (TTV) a single stranded DNA virus (ssDNA) was first reported in 58 years old patient with recent history of blood transfusion (35 Units) suffering from non A-G hepatitis in 1997 from Japan (Nishizawa *et al.*, 1997 and Okamoto, *et al.* 1999 ). Human Torgue teno virus (TTV) is also known an orphan virus (viruses that are not associated with any disease but may cause pathogenicity) (Bostan & Bokhari, 2013). TTV was proposed as a member of a new family named “*Circoviridae*” and its genus is “*Anelloviridae*” (Okamoto *et al.*, 1998). The genome of TTV exhibits an astonishingly high genetic diversity and is classified into nearly 39 genotypes (Asim *et al.*, 2010). TTV a circular structure and negative polarity of the genome, a single-stranded DNA 3,852 nt long. Moreover, the buoyant density in CsCl (1.31-1.34 g/cm3), the particle size as determined by filtration (30-50 nm) (Cross, 2000). TTV particles in serum, bile and feces have the same density; the genome of TTV is a single-stranded and circular DNA of approximately 3.9 kb. The genomic length varies depending on genotype, and measures 3,853 bases in the prototype (Okamoto *et al.*, 1999).

TTV genome includes two regions: a coding region (N22) and an untranslated region (UTR). The UTR is located at 3075-3853 nucleotides and 1-352 nucleotides occupying approximately 30% of the genome (Ukita *et al.*, 2000). Coding region consist of 6 ORFs with open reading frames (ORF1-ORF6) (Yokoyama *et al.*, 2002).

TTV has been found in many body fluids including saliva, milk, tears and fecesl, Parenteral , fecal-oral ,mother to fetus and sexual routes have been suggested for TTV transmission (Ataei *et al.*, 2012). 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 (Ataei et al., 2012). TT viruses (TTV) are ubiquitous in nature and have been demonstrated in more than 90% of serum samples from healthy individuals where they persist over time (Mokhtarzadeh et al., 2009).

Sudan is classified among countries with a high hepatitis B surface antigen (HBsAg) endemicity of more than 8%. Exposure to HBV infection ranges from 47% to 78% with a hepatitis B surface antigen (HBsAg) seroprevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan (Mudawi, 2008). The historical background of TTV discovery suggested that TTV is a hepatitis virus and the early study focus on the relation between HBV/TTV, and they found that TTV is widely prevalence in HBV patient, the association between HBV/TTV it not clear established yet (Bostan & Bokhari, 2013).

According to the lack of data about the prevalence of TTV in Khartoum and Sudan generally, this study was conducted to generate preliminary infection rate of TTV in order to determine the existence of TTV DNA infection and the prevalence among healthy blood donors of TTV among healthy blood donors.

**Material and Methods**

This study was carried out on blood donors admitted to blood bank at soba Hospital during October to November 2012. After explaining the purpose of the study, and after obtaining the verbal approval, data were collected from each volunteer by interviewing questionnaire. Collected data included, gender, date of sample collection, age, place of sample collection and health status. The obtained data were saved for statistical analysis. A total of 81 blood samples (5ml in EDTA) from blood donors who are included in this study. Blood samples were centrifuged at 5000 rpm for 5 minutes then serum were collected and stored at -20 °C till test was performed.

TTV DNA was extracted from serum using Viral Gene-spin™ Kit RNA / DNA Isolation Kit. Intron Biotechnology Company, Korea. Following the
manufacturers procedure, DNA pellets were then collected and stored in 4 C° for polymerase chain reaction technique

The PCR was performed by processing the extracted DNA from serum with primers that are specific for UTR Region of TTV, the primer consisting of 5’-GCTACGTCACTAACCACGTG-3’ and 5’-CTCCGGTGTTGTAAMACTCACC-3’ (T801, and T935) as designed by (Takahashi et al., 1998). 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 (Esco Swift) as follow: initial denaturation at 94ºC for 5 minutes followed by 55 cycles Denaturation at 94ºC for 20 s, annealing at 60ºC for 20 S and template extension at 72ºC for 30 s .The cycle was repeated 55 times., with a final extension 72ºC for 5 minutes.

10μl of the amplified product was subjected to direct analysis by Gel Electrophoresis in 2% agarose; the gel was prepared by adding 2g of agarose to 100 ml 1X TBE Tris Borate EDTA buffer and 1.5 μL ethidium bromides. The product was visualized by staining with ethidium bromide using UV gel documentation system. A 199 bp product was amplified with above (T801, and T935) primer.

**Statistical analysis:**

Collected data were analyzed using the Statistical Package for Social Science (SPSS); Test of proportion and Correlations analysis were used. P value >0.05 was considered no significant.

**Result and Discussion:**

It was not known how common TTV was in Khartoum. Thus; we initiated our TTV research by testing, with PCR methods (UTR PCRs) to determine the prevalence in blood donors in the Khartoum population.
83% blood donors showed positive TTV in their serum samples, and 17% blood donors showed negative TTV in their serum samples (Table 1), by conventional PCR reaction to detect the UTR region.

Fig 1: Agarose gel (2%) electrophoresis showing detection of TTV by convention PCR technique in DNA extracted from Sudanese blood donors’ sera. M = DNA ladder 100 bp marker, +ve control in lane 2, lane 3-8 +ve samples (1-6).

The high prevalence in this study is comparable with figures from other healthy populations in Africa (South African (84%), Tanzania (74%), Gambia (83%), Ghana (88%) and Egypt (85%)) and in developed countries around the world 34 - 90% (Heidi, 2003). The high prevalence of TTV DNA carrier state found in our study compared to others may be attributed to the population density, lifestyle, standard of living and mode of transmission. This suggests that routes other than the parental route must play a role in TTV transmission. Evidence for the presence of TTV in feces and saliva indicates that horizontal transmission via the fecal-oral route and aerosols may be important (Heidi, 2003).

The high frequency of the virus in this study compared to the lower frequencies reported in other studies in world could be correlated to the methodology and the PCR primers used, and may be also attributed to the extreme heterogeneity of its genome making detection dependent on the viral DNA segment targeted for amplification. This has an enormous impact on PCR sensitivity, (Takahashi et al., 1998; Salakova et al., 2004).
Table 1: Detection of TTV DNA in serum of healthy blood donors in Khartoum state using PCR to detect UTR region during 2013

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total tested</th>
<th>No. – ve</th>
<th>No. + ve</th>
<th>% - ve</th>
<th>% + ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>81</td>
<td>13</td>
<td>68</td>
<td>17%</td>
<td>83%</td>
</tr>
</tbody>
</table>

Due to its higher conservation, the UTR of the TTV genome is much more suitable for primer design (Bendinelli et al., 2001). Indeed, generally UTR PCR has proven satisfactory at detecting most if not all the TTV genotypes currently recognized and compared to ORF1 PCR (Mokhtarzadeh et al., 2009). Moreover, the ubiquitous nature of the virus raises the speculation whether the virus is pathogenic, opportunistic, a cofactor of other infections or a modulator of immunity that can promote other microbes to be infectious (Maggi et al., 2003). The high prevalence of TTV in general population, may complicate linking TTV to hepatic disease and other pathologic states (Maggi et al., 2003). Attempts to correlate TTV with liver disease need further studies that deal with viral load quantification, genetic characterization and detection of the virus in liver tissue.

This unusual feature among viruses aroused the proposal that TTV might be a commensal virus or part of human micro flora. Another major complication is the extreme heterogeneity of TTV genome, its divergent genogroups (1-5), and genotypes (40) each of which possesses distinct biologic properties and pathogenic potentials (Omar et al., 2006)

**Conclusion:**

Incidence and extent of TTV in Sudan was documented through detection of TTV DNA in blood donors samples indicating high prevalence among blood donors, generally this finding are useful for future study seen their little available information in Sudan.TT virus is still an open problem and it requires careful studies with the use of the most modern techniques of molecular biology.
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