Transfusion transmitted virus in screened United Arab Emirates blood donors

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ABSTRACT

Objective: To investigate the rate of infection caused by Torque teno virus (TTV) in United Arab Emirates' (UAEs) healthy population as a pilot study in detecting TTV DNA in 100 healthy blood donors.

Methods: We randomly choose a total of 100 healthy blood donors who attended Zayed Military Hospital, Abu Dhabi, UAE from January 20 to May 30, 2005. We carried out a real-time polymerase chain reaction (PCR) test to detect TTV DNA.

Results: Real-time for TTV was positive in 75 (75%) donors. Eight (73%) non-UAE donors were TTV positive while 67 (75%) were UAEs. Among these donors, 72 (77%) were males and 3 (50%) were females.

Conclusion: Our results demonstrated a high prevalence of TTV in UAE.

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Torque teno virus or TT virus (TTV) is a naked, circular, non-enveloped virus[1] with a negative-stranded DNA genome of 3,818-3,853 nucleotides, suggested to be a member of circoviridae family.[2-4] It was first identified in 1997 and named after the initials index of Japanese patient who was suffering with post transfusion hepatitis of unknown etiology.[5] Zuckermann,[6] later described it as "transfusion transmitted virus" abbreviated as TTV.

The TTV chronically infects healthy individuals of all ages in different populations worldwide.[7,8] Studies found seroprevalences of TTV to be approximately 30-93% in normal healthy population from different parts of the world, including developed and developing countries.[7-13] Genoprevalence rates of TTV DNA in the sera of normal blood donors varies geographically, such as 12% in Japan and 36% in Thailand.[7-13] In addition, reported rate for TTV DNA was 92% among healthy Japanese subjects,[7-13] and 1% among the United Kingdom and United States subjects.[14,15] In the UAE detection rates are 34.9% in healthy nationals and 89.1% in healthy non-nationals.[16]

The TTV being a DNA virus exhibits an astonishingly large amount of genetic diversity with more than 40 genotypes from classified 5 major phylogenetic groups (G1 to G5).[17] The evolutionary distance between classified genotypes is separated by more than 30% divergence at nucleotide level in N22 region of ORF1. The prevalence of most common genotypes G1 and G2 is similar all over the world, while other genotypes is scarce and not conclusive. Polymerase chain reaction (PCR) using primers, which target the ORF1 region, can detect only TTV

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genotype 1-6 of group 1, but PCR primers designed for NCR can detect nearly all genotypes.[18,19]

Transmission of TTV has not yet been elucidated very clearly even though numerous studies have suggested that the potential transmission via transfusion of contaminated blood and blood products is the most common route of TTV infection.[14] Studies detected transmission of TTV in saliva,[20] breast milk,[21] semen[22] and vaginal fluid.[23] There is evidence that TTV is excreted into feces of infected individuals, suggestive of possible fecal-oral transmission as well.[24] It is also suggestive of the possible involvement of other specific environmental factors in the acquisition of TTV infection.[25]

Several reports state the association of TTV with non A-E transfusion–acquired hepatitis. Acute resolving and chronic persistent hepatic infections have been recognized among TTV infected humans.[26] Recent clinical studies suggest that TTV is pathogenic for the liver and may trigger fulminant hepatic failure. Furthermore, some studies concluded that TTV DNA in serum does not affect hepatitis C virus (HCV) infection or liver damage caused by HCV. [27]

In our present study, we performed a previously published real-time PCR assay for the detection of TTV DNA. We used the high pure viral nucleic acid kit (Roche Applied Systems, Germany) for the isolation of viral nucleic acids. Our study aim to investigate the rate of TTV infection among the UAE's healthy population as a pilot study by detecting TTV DNA in 100 healthy blood donors from different populations of UAE.

Methods.

Sample collection. A total of 100 healthy blood donors were randomly selected, who attended Zayed Military Hospital, Abu Dhabi, UAE from the January 20 to May 30, 2005 were included in the study. Zayed Military Hospital is a tertiary medical center, and it covers in and out patients. A 10 mL venous blood sample, obtained from each participant, was separated within 3 hours of collection and stored at -80^{0}C until further processing. Donors were informed about the study but no consent was obtained.

Sample rejection criteria. All subjects underwent short interview regarding history of surgical intervention, blood transfusion, intravenous drug abuse, parenteral treatment or tattoo making. Any subject having one of above-mentioned things was rejected. All subjects were screened for HIV 1 and 2 antibodies and antigens (HIV1/2 Ag/Ab), hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBc Ab), hepatitis C virus antibody (HCV Ab), and human T-cells lymphotrophic virus type 1 and 2 antibodies (HTLV1/2 Ab). Any positive subject for any one of these above-mentioned markers was rejected from our study.

Nucleic acid extraction. Nucleic acid extraction was done using the high pure viral nucleic acid kit (Roche, Germany) following the manufacturer's procedure. Purified nucleic acid was eluted in 50 μl elution buffer and stored at -80^{0}C.

Primers and probes. Specific primers and probes used were published previously,[28] in which specific oligonucleotide primers, derived from the ORF2 region of TTV, were employed. This conserved region may be the only segment that allows the design of primers that can be expected to amplify most TTV strains. Oligonucleotides deduced from the published sequence of TTV genome TA278 (GenBank accession no. AB008394) were used (**Table 1**). With these oligonucleotides, a 157-nucleotide amplification product was generated. For detection of the target sequence, hybridization probes (TIB MOLBIOL, Berlin, Germany) were labeled with LC Red 640 at the 5' end and with fluorescein at the 3' end.

Real-time polymerase chain reaction on the Light Cyler (LC) instrument. Real-time PCR was

Table 1 - Primers and probes used for real-time polymerase chain reaction assay.

Primers and probes	GenBank accession no.
TTV forward (5-CCGAATGGCGAGTTTTCCA)	AB008394
TTV reverse (5-TTTTCAGAGCCTTGCCCATAG)	AB008394
TTV FL (5-CGAATTGCCCCTTGACTTCGGTGTG)	AB008394
TTV LC (5-AACTCACCTTCGGCACCCGCCCTC)	AB008394

Table 2 - Prevalence of TTV DNA in UAE and non-UAE populations in the UAE in relation to gender.

Subjects	UAE			Non-UAE			Total		
	Number tested	Positive for TTV	(%)	Number tested	Positive for TTV	(%)	Number tested	Positive for TTV	(%)
Males	84	65	(77)	10	7	(70)	94	72	(77)
Females	5	2	(40)	1	1	(100)	6	3	(50)
Total	**89**	**67**	**(75)**	**11**	**8**	**(73)**	**100**	**75**	**(75)**

UAE – United Arab Emirates, TTV - transfusion transmitted virus

performed on the LC instrument (Roche, Germany). All samples were run with the LC Fast Start DNA master hybridization probes kit (Roche, Germany). The PCR master mix contains 2 μl Fast Start master DNA hybridization probes reaction mix, 1.6 μl MgCl (final concentration: 3 mM), 0.2 μl each (final concentration: 0.5 μM of the 3 mM), 0.2 μl each (final concentration: 0.5 μM of the 0.2 μ M) of TTV FL and TTV LC hybridization probes, 0.2 μl each (final concentration: 0.2 μM) of Neo-LC Red and Neo-FL of neo-hybridization probes and PCR-grade sterile water (each 10.2 μl) to a final volume of 15 μl. A 5 μl aliquot of extracted sample was added to 15 μl of PCR master mix in each LC glass capillary. After this, LC capillaries were sealed, inserted into the specially designed LightCycler Carousel (Roche, Germany), and centrifuged with 3000 g for 15 seconds. Finally, the LC carousel was placed into the LC instrument. The cycling protocol was run as follows: one cycle of 95^{0}C for 7 minutes followed by 65 cycles consisting of denaturation for 1 second at 95^{0}C, annealing for 10 seconds at 64^{0}C, and elongation for 25 seconds at 72^{0}C. After the final cycle, the melting curve was started at 50^{0}C for 1 minute and the thermal chamber temperature was slowly (0.2 C/s) raised to 85^{0}C and the fluorescence was measured stepwise. The capillaries were then cooled for 2 seconds at 40^{0}C. Fluorescence curves were analyzed with the LC software (version 3.5.3). The calculation of crossing points was carried out by the automated second derivative maximum method. Channel F2 was selected for the target sequence.

Statistical analysis. Using Chi-square test, statistical analysis was carried for comparison of proportions between 2 groups. Differences were considered to be statistically significant at $p<0.05$.

Results. **Table 2** summarizes all the results of the total samples studied. Only 6 (6%) of the samples were for female donors and 89 (89%) were for UAE national donors. The age of the donor's ranged from 18-65 years. All 100 donors were negative for HIV1/2 Ag/Ab, HBsAg, HBc Ab, HCV Ab, and HTLV1/2 Ab.

Real-time for TTV was positive in 75 (75%) donors. Eight of 11 (73%) non-UAE national donors were TTV positive, 67 of 89 (75%) UAE donors were TTV positive, 72 of 94 (77%) donors were TTV positive among the males and 3 of 6 (50%) donors were positive among the females.

The rate of TTV detection by gender (**Table 2**) was higher in males than in females; this might be due to the fact that more male subjects were studied (94%). The TTV rate was detected more among UAE national subjects (**Table 2**); again, this might be due to the fact that more UAE subjects were studied than non-UAE.

Discussion. The wide distribution of TTV, with a high frequency of viremia in adults, and the world prevalence of TTV is very variable depending on the set of primers used in the PCR assay.[18] During our study period, the total number of healthy blood donors was 646. In our random subject (100 samples) the TTV rate was 75%. We noticed that the TTV infection rate among UAE healthy national was higher (75%) than that of healthy non-UAE nationals (73%). This could be attributed to the population density, life style and our subject size. By comparison, the prevalence of TTV in blood donors in the United States ranged from 1-10%.[14,29] Similarly, in European countries, TTV prevalence rate in healthy donors ranged from 1-13%.[15,30] Other reports from other countries reported the prevalence of TTV of healthy blood donors were as follows: Italy 50%, Japan 92%, Mongolia 62%, Thailand 42.9%, Taiwan 7.53% and Korea 17.6%.[12,30-32] In UAE, TTV prevalence rate in a previous study was 34.9%.[16]

When we analyzed the results with respect to gender differences, our results (**Table 2**) showed that

TTV DNA was higher ($p<0.05$) in healthy males (77%) compared to females subjects (50%). These results corroborate previous reports.[33]

In conclusion, the results obtained in the present study indicate the presence of TTV in healthy individuals in the UAE. Our results demonstrate a high prevalence of TTV in UAE. The TTV infection rates are higher in males than females. Our data, as well as the results of other studies, show that optimization of the primers set for more standard TTV detection is still needed. Further virological and epidemiological studies are needed with respect to genotyping, genetic grouping, and TTV load testing to better understand the clinical and natural course of TTV infection.

There is no doubt that the astonishingly high prevalence of TTV worldwide with minimal or no disease association is perplexing. Hopefully, investigating the many fundamental questions that remains to be answered will lead to the identification of hitherto unsuspected etiological links and ultimately will perhaps reveal further avenues for significant public health improvements, such as in the field of transfusion and organ transplantation medicine.

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