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# Primers Designed for Detection of TT Virus Also Detect SEN Virus

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**Abstract:** TTV was first identified in 1997. Recently SEN Virus (SENV), was discovered which is distantly related to TTV with about 55% sequence homology, so consensus primers (T801 and T935) already used for the detection of TTV may also detect SEN virus. DNA extracted from serum was subjected to PCR using T801 and T935 primers and the amplified segment was subjected to sequencing. We demonstrated that the prevalence of the virus in serum samples of blood donors in Iran was 79.5% (105/132). The nucleotide sequence revealed up to 94% homology with isolates of TT virus and up to 92% homology with isolates of SEN virus. This study shows high prevalence of TT viruses in Iran which is similar to other countries. The results suggest that PCR primers for the UTR region of TTV, which are commonly used in prevalence studies, can also amplify SEN virus. Thus, the reported prevalence using such primers should refer to the family of TTV (TTV and related viruses), which includes TT virus and SEN virus.

**Key words:** TT virus, SEN virus, PCR, TTV, DNA virus

## INTRODUCTION

TT Virus (TTV) is an unenveloped negative single-stranded circular DNA virus, with a genome of about 3.8 kb and a virion diameter of 30-32 nm (Itoh *et al*., 2000; Miyata *et al*., 1999; Mushahwar *et al*., 1999). TTV has been classified recently, together with related Torque teno minivirus, into a new floating genus called *Anellovirus* (Devalle and Niel, 2005). It was first identified by Nishizawa *et al*. (1997) means of Representational Difference Analysis (RDA) in the plasma of a Japanese patient (initials T.T.) with posttransfusion non-A-G hepatitis (Nishizawa *et al*., 1997). There is no evidence of its association with any liver or other specific disease to date. However, TTV is a virus with a great genetic heterogeneity and it is speculated that certain genotype(s) may be capable of causing hepatitis (Itoh *et al*., 1999; Okamoto *et al*., 1999a; Okamoto *et al*., 1999b). Use of different PCR primers and conditions in the determination of TTV prevalence has led to discrepant results. At first the TTV PCR systems established by Nishizawa *et al*. (1997) and Okamoto *et al*. (1998) had low sensitivity and were unable to detect all isolates so at that time it seemed the true prevalence was under estimated. To resolve this problem, Takahashi *et al*. (1998) established a PCR system with primers (T801 and T935) designed for a very conserved Untranslated Region (UTR) of the viral genome that was 10-100 times more sensitive than the previous systems and could detect all known genotypes of TTV. Using these primers, they reported that TTV DNA was identified in the sera of 92% of 100 healthy individuals in Japan (Takahashi *et al*., 1998). PCR systems with primers designed for the UTR are more sensitive than the systems with the primers designed for N22 and other regions of the viral genome and indicated high prevalence (>90%) of TTV infection in general population of many countries world-wide (Itoh *et al*., 1999; Takahashi *et al*., 1998; Abe *et al*., 1999).

Recently, a novel single-stranded circular DNA virus of approximately 3.8 kb, designated as the SEN (the initials of the patient from whom the virus was recovered) virus (SENV), was discovered in the serum of an intravenous drug abuser infected with human immunodeficiency virus (Primi *et al*., 2000). SENV is distantly related to TTV with which it shares a similar genome organization but only about 55% sequence homology (Tanaka *et al*., 2001). It belongs to the superfamily of TTV- related viruses (Sagir *et al*., 2004).

There is little information about the circulation of TTV in the general population of Iran. Primarily this study was designed to determine the prevalence of TTV in voluntary blood donors in Isfahan, Iran. Although, there is a lot of information about the homology in the genome of the 2 viruses (TTV and SENV), the consensus primers (T801 and T935) used for detection of TTV may also detect SEN virus.

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## MATERIALS AND METHODS

**Serum samples:** Serum samples were collected from 132 voluntary blood donors at the Isfahan blood transfusion organization and stored at -20°C until tested. The donors ranged in age from 18-82 years, 4 were positive for HBs-Ag and 6 were positive for Anti-HCV antibodies.

**DNA extraction:** Viral DNA was extracted using phenol/chloroform after treatment of 200 μL of serum with 0.5 mg $mL^{-1}$ of proteinase K in the presence of 0.2 M NaCl, 0.25% Sodium Dodecyl Sulfate (SDS), for 2 h at 65°C. The pellet was dried and resuspended in distilled water or TE (Tris-HCl buffer (10 mM, PH 8.0) containing 1 mM EDTA) solution after precipitation with ethanol.

**PCR amplification:** The T801 and T935 primers were used for amplification of a highly conserved noncoding region (UTR) of the viral genome (Takahashi *et al*., 1998). The expected product size was 199 bp (Takahashi *et al*., 1998). DNA was amplified by a single round of PCR performed with 3 μL of DNA in a 25 μL reaction mixture containing 1 U of Smar *Taq* DNA Polymerase (Cinnagen, Iran), 0.4 μM of each primers, 240 μM of each dNTPs, 20 mM of Tris-HCl, 3 mM $MgCl_2$, 50 mM KCl and 20 mM Ammonium Sulfate. Thermal cycling conditions were as follows: denaturation of 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 20 sec, annealing at 57°C for 25 sec and extension at 72°C for 30 sec. The amplification program was followed by a final extension step at 72°C for 5 min. PCR products (10 μL) were loaded on a 2% agarose gel (Sigma, Germany) containing ethidium bromide and electrophoresed.

**DNA sequencing and computer analysis of TTV sequences:** To confirm the presence of TTV DNA detected in specimens, amplicons were sequenced (SEQLAB, Germany). A WU-BLAST-2 search of the determined sequences against a nucleotide sequence database (EMBL, European Bioinformatics Institute) was performed.

## RESULTS

In PCR a 199 bp product was detected in 105 out of 132 (79.5%) serum samples tested (including all of the 10 hepatitis positive sera). Alignment of the sequence of the 199 bp PCR product (Accession number DQ133465) against the prototype TTV (TA278, Accession number AB008394) (Nishizawa *et al*.,1997) showed homology of 87% (Fig. 1). However, a Blast search showed higher levels of homology with other isolates of TTV (92-94%)

Table 1: Specifications of 8 TTV with highest homology with 199 bp product (DQ133465) using T801 and T 935 primers

| Alignment | DB:ID | Source | Length | Score | Identity (%) | Positives (%) | E() |
|---|---|---|---|---|---|---|---|
| 1 | EM_VI:AF159224 | TT virus strain TTmBJ unknown gene. | 194 | 881 | 94 | 94 | 4.5e-32 |
| 2 | EM_VI:AF159225 | TT virus strain TTm5 unknown gene. | 194 | 857 | 93 | 93 | 5.5e-31 |
| 3 | EM_VI:AJ620226 | TT virus ORF1 and ORF2, isolate tth4 | 3772 | 859 | 92 | 92 | 7.9e-31 |
| 4 | EM_VI:AJ620219 | TT virus ORF1 and ORF2, isolate tth9 | 3755 | 852 | 92 | 92 | 1.6e-30 |
| 5 | EM_VI:AJ620220 | TT virus ORF1 and ORF2, isolate tth16 | 3756 | 852 | 92 | 92 | 1.6e-30 |
| 6 | EM_VI:AJ620221 | TT virus ORF1 and ORF2, isolate tth17 | 3757 | 852 | 92 | 92 | 1.6e-30 |
| 7 | EM_VI:AJ620225 | TT virus ORF1 and ORF2, isolate tth31 | 3757 | 852 | 92 | 92 | 1.6e-30 |
| 8 | EM_VI:AJ620222 | TT virus ORF1 and ORF2, isolate tth25 | 3758 | 852 | 92 | 92 | 1.6e-30 |

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TTV-Isfahan:  1 GCTACGTCACTAACCACGTGACTCCCGCAGGCCAACCAGAGT-CTATGTCGTGCACTTCC  59
TA278:        6 GCTACGTCACTAACCACGTGACACCCACAGGCCAACC-GAATGCTATGTCATCCATTTCC  64

TTV-Isfahan: 60 TGGGCATGGTGTACGTGATAATATAAAGCGGTGCACTTCCGAATGGCTGAGTTTTCCACG  119
TA278:       65 TGGGCCGGGTCTACGTCCTCATATAAGTAAGTGCACTTCCGAATGGCTGAGTTTTCCACG  124

TTV-Isfahan: 120 CCCGTCCGCAGCGGCAACACCACGGAGGGTGATCTCCGCGTCCCGAGGGCGGGTGCCGTA  179
TA278:       125 CCCGTCCGCAGCGGTGAAGCCACGGAGGGAGATCTCCGCGTCCCGAGGGCGGGTGCCGAA  184

TTV-Isfahan: 180 GGTGAGTTTACACACCGCAG  199
TA278:       185 GGTGAGTTTACACACCGAAG  204
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Fig. 1: Sequence alignment of 199bp PCR product (TTV-Isfahan, DQ133465) against prototype virus (Japanese TTV isolate, Nishizawa *et al*., 1997) i.e. TA278 (Accession number AB008394) (EMBL, European Bioinformatics Institute, WU-BLAST-2)

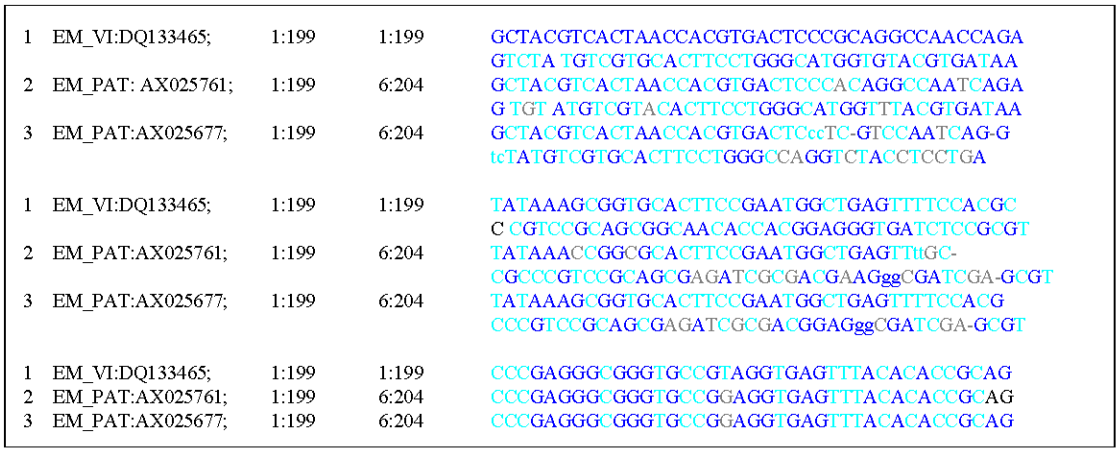


Fig. 2: Sequence alignment of 199bp PCR product (DQ133465) against 2 strains of SENV (EMBL, European Bioinformatics Institute, WU-BLAST-2). Nucleotides complementary to primers are underlined

GCTACGTCACTAACCACGTGGGGAAGCTTTCATACCAGGAGAGCCAGGCCTTGACCCTGCATTTACAGGCTCTGAG
AAATTAGAGACATGGTCTACTCCAATATCTGAGATCCAGAGAGTGAAAGCCCAAAGTCTGGGTGAGTTTACACACC
GNAG

Fig. 3: The sequence of 156bp band observed in gel electrophoresis of PCR products using T801 and T935 primers (Accession number DQ133466)

(Table 1) and with isolates of SEN virus (87-92%), for example, the homology with isolates AX025761 and AX025677 were 87 and 89%, respectively. Analysis of the sequence data on SEN viruses AX025761 and AX025677 indicated that primers T801 and T935 were complementary to bases 6-25 and bases 185-204, respectively (Fig. 2).

In addition to the expected 199 bp product, a product of 156 bp (Fig. 3) was observed from 79 samples. Sequencing of 156 bp products (Accession number DQ133466) revealed high levels of homology with human and mouse genomic sequences (96% identity with human DNA sequence from clone RP11-431O22 on chromosome 13, containing the 5' end of the gene for chondromodulin I precursor (CHM-I), a novel gene, the PCDH8 gene for protocadherin 8 and 4 CpG islands).

## DISCUSSION

Primers T801 and T935 were designed for amplification of very conserved noncoding region (UTR) of TT virus by Takahashi *et al.* (1998). Using these primers revealed high prevalence (>90%) of TTV in general populations around the world (Takahashi *et al.*, 1998; Abe *et al.*, 1999). However, at that time SENV had not been discovered. It was first discovered by Primi *et al.* (2000) and its sequence was published recently (Tanaka *et al.*, 2001). In this study, it was shown that the above primers will also amplify from SENV a product of the same size (199 bp) as that from TTV. Thus, it is possible that previous reports on the prevalence of TTV may have included people positive for SENV rather than TTV. Accurate diagnosis of TTV and SENV infections and studies on the prevalence of these viruses require virus-specific PCR primers.

Almost 80% of serum samples from blood donors in Isfahan, Iran were positive for TTV family (TTV or related viruses). This is similar to the prevalence shown in other countries. The detection of these viruses in such a large proportion of the population supports the view that they are unlikely to be associated with disease. However, the pathogenicity of these viruses especially TTV remains to be resolved.

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