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LETTER

New prevalence estimate of Torque Teno virus (TTV) infection in healthy population and patients with chronic viral hepatitis in Jiujiang, China

Dear Editor,

Torque Teno virus (TTV) is a nonenveloped human DNA virus that was isolated from the serum of a patient with transfusion-transmitted hepatitis with unknown etiology in 1997 (Nishizawa et al., 1997). TTV is the first human virus with a single-stranded circular DNA genome to be identified, and is recently classified as the *Alphatorquevirus* genus of the *Anelloviridae* family by the International Committee on Taxonomy of Viruses (ICTV) (King et al., 2011). TTV shows very high genetic variability for a DNA virus (Hussain et al., 2012; Chen et al., 2013). Thus far, five main genetic groups (groups 1–5) including at least 39 genotypes have been identified based on phylogenetic analysis (Peng et al., 2002; Okamoto et al., 2004; Hussain et al., 2012; Mi et al., 2014). Two distinct PCR methods have primarily been used for investigating the prevalence of TTV infection. The earlier N22-PCR, with primers derived from the N22 region within ORF1, can detect only a portion of TTV variants mainly representing genetic group 1 TTVs (genotypes 1–6) (Okamoto et al., 1999; Peng et al., 2002; Okamoto et al., 2004; Hussain et al., 2012), and this method is mostly used for investigating TTV prevalence in the Chinese population (Zhao et al., 2002). In contrast, the untranslated region PCR (UTR-PCR) can detect essentially all known TTV strains reported (Takahashi et al., 1998; Peng et al., 2002; Okamoto et al., 2004).

In the current study, UTR-PCR and N22-PCR were utilized for estimating the prevalence of new TTV infections by examining serum samples obtained from healthy infants, healthy adults, chronic viral hepatitis B patients, and chronic viral hepatitis C patients, in an attempt to more accurately elucidate the prevalence of TTV infections in the general population and in patients with liver disease in Jiujiang, China.

A cohort of serum samples from 80 patients with confirmed diagnoses of chronic hepatitis B, 80 patients with confirmed diagnoses of chronic hepatitis C collected in two infectious hospitals in Jiujiang, 98 healthy blood donors and 86 healthy infants in Jiujiang Women and Children's Hospital, respectively, was enrolled in the present study. All of the individuals involved in the current study were living in Jiujiang, Jiangxi Province, and did not have a history of blood transfusion before the serum samples were collected. Informed consents were signed by the individuals or their custodians involved in this study.

UTR-PCR used in this study was a modification of a previously described method (Okamoto et al., 1999; Peng et al., 2002). Primers used for N22-PCR were previously reported by Okamoto et al (Okamoto et al., 1999; Rivanera et al., 2009; Hussain et al., 2012).

To ascertain the specificity of the PCR products, DNA sequences of two randomly selected PCR products were determined. The sequence homology between the PCR products and TTV prototype TA278 was compared and the determined nucleotide sequences of TTV were found to be homologous to TTV prototype TA278 (accession number AB017610), with 89.9% or 94.7% sequence identities in the corresponding UTRs. These results indicate that the positive DNA obtained by UTR-PCR was specifically amplified TTV DNA.

The prevalence of TTV infection in different populations was evaluated by UTR- and N22-PCR. As shown in Table 1, TTV viremia was detected by UTR-PCR in 96 (98.0%) of 98 healthy adults, 80 (100%) in 80 patients with chronic viral hepatitis B (PCVHB), and in 78 (97.5%) of 80 patients with chronic viral hepatitis C (PCVHC), while it was detected in 37 (37.8%) of 98 healthy adults, 28 (35.0%) of 80 PCVHB, and 34 (42.5%) of 80 PCVHC by N22-PCR. There was no significant difference in the prevalence of TTV viremia in healthy adults and patients with liver disease as detected by UTR-PCR and N22-PCR ($p > 0.05$). Furthermore, there was no significant difference in the positive rate of TTV DNA observed among healthy adults, PCVHB, and PCVHC ($p > 0.05$).

As shown below in Table 1, TTV viremia was detected by UTR-PCR in 47 (54.7%) of 86 healthy infants, while it was detected in 15 (17.4%) of 86 healthy infants by N22-PCR, both significantly lower than that obtained

Table 1. The positive rate of TTV infection was detected by UTR- and N22- PCR in healthy blood donors, hepatitis patients and infants.

Group (age)	UTR-PCR Positive/total (%)	N22-PCR Positive/total (%)	*p*
Adults (20-56 years)	96/98 (98.0)[a1]	37/98 (37.8)[a2]	< 0.01
PCVHB (28-51 years)	80/80 (100)[b1]	28/80 (35.0)[b2]	< 0.01
PCVHC (21-66 years)	78/80 (97.5)[c1]	34/80 (42.5)[c2]	< 0.01
Infants (5-358 days)	47/86 (54.7)	15/86 (17.4)	< 0.01
< 1 month	0/19 (0)	0/19 (0)	
< 1-6 months	9/27 (33.3)[d1]	2/27 (7.4)[d2]	< 0.01
< 7-12 months	38/40 (95.0)[e1]	13/40 (32.5)[e2]	< 0.01

PCVHB or PCVHC indicates patients with chronic viral hepatitis B and chronic viral hepatitis C, respectively. *p*-values listed in this table represented the comparison between UTR- and N22-PCR in the same group. The positive/total ratio of infant group (86) vs. adult group (98), PCVHB group (80) and PCVHC group (80) were compared, using UTR- and N22-PCR methods, and all showed $p < 0.01$. Other $p < 0.01$ comparisions:d1 vs. a1, d1 vs. b1, d1 vs. c1, d1 vs. e1, d2 vs. a2, d2 vs. b2, d2 vs. c2, d2 vs. e2. Statistical significance in this table was analyzed by the $\chi 2$ test.

from the healthy adults and chronic hepatitis patients ($p < 0.01$). Furthermore, among 19 infants from whom blood samples were obtained less than 1 month after birth, no TTV DNA was detected by UTR-PCR or N22-PCR. However, positive TTV DNA obtained by UTR-PCR was detected in 9 (33.3%) of 27 infants at the age of 1–6 months, and 38 (95.0%) of 40 infants at the age of 7–12 months. Similarly, TTV DNA was detected by N22-PCR at a positive rate of 2 (7.4%) in the 1–6 months group and 13 (32.5%) in the 7–12 months group. Interestingly, the incidence of TTV infection in the infants at the age of 7–12 months was comparable to those among healthy adults and chronic hepatitis patients ($p > 0.05$).

In the absence of a serological system, detection of TTV DNA by PCR thus far has been the only confirmed method for TTV diagnosis. In China, TTV infection was found in 39.2%–48% of chronic hepatitis patients compared to 16.7%–52.0% of blood donors by N22-PCR or PCR utilizing primers in coding regions (Ren et al., 1999; Chen et al., 2001; Peng et al., 2002; Wang et al., 2003). Relatively few epidemiological surveys utilizing UTR-PCR for detecting TTV have been reported, especially in healthy adults and infants. In the present study, UTR- and N22-PCR were performed to test and compare the positive rate of TTV DNA in serum samples obtained from healthy infants and adults, PCVHB, and PCVHC.

Our results suggest that TTV is extremely common in the general Chinese population, including infants, providing further evidence that TTV is highly prevalent in the general population worldwide (Mancuso et al., 2013). Furthermore, the overall prevalence of TTV infection based on UTR-PCR in healthy infants, healthy adults, and patients with liver disease was almost 2–3 times higher than that obtained by N22-PCR ($p < 0.01$, Table 1), which highlights both the considerable influence of the PCR primers on the detection of TTV DNA and the benefits of using UTR-PCR to establish the true overall prevalence of TTV infection. On the other hand, our results also indicate the lack of a clear association of TTV infection with chronic viral hepatitis, which strongly suggests that TTV has little potential for causing hepatitis. However, the virulence and pathogenesis of the different genotypes or strains of TTV remain unclear and require further investigation.

In three different age groups of infants, all samples collected from infants before 1 month of age were negative for TTV DNA as detected by both UTR- and N22-PCR, while an increasing positive rate in the older infant age groups was observed. TTV infection in infants at 7–12 months of age was comparable to that of healthy adults and patients with liver disease living in Jiujiang city. This result was also observed in other studies in different countries (Davidson et al., 1999; Ohto et al., 2002; McLaughlin-Drubin and Munger, 2008). The age-related increase in TTV infection implied the existence of a very efficient means of transmission, the more powerful fecal oral route transmission other than the parenteral transmission. Thus, the real incidence of TTV infection, as well as its transmission mode and pathogenesis need to be further taken into account.

FOOTNOTES

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