BRIEF REPORT

High Carriage Rate of TT Virus in the Cervices of Pregnant Women

Paul K. S. Chan,[1] Wing-Hung Tam,[2] Winnie Yeo,[3] Jo L. K. Cheung,[1] Sheng Zhong[3] and Augustine F. Cheng[1]

Departments of [1]Microbiology, [2]Obstetrics and Gynaecology, and [3]Clinical Oncology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

Prevalence studies of the recently identified TT virus (TTV) have suggested that parenteral transmission is a common route of infection, but other routes also appear likely. In this study, a high rate of cervical carriage (66%) of TTV DNA was found by polymerase chain reaction, which suggests that perinatal and sexual transmission is possible.

A novel DNA virus, TT virus (TTV), was recently identified from a Japanese patient with posttransfusion hepatitis of unknown etiology [1]. The clinical significance and natural history of TTV infection are still a mystery. Viral DNA has been detected in patients with non-A–G hepatitis, cirrhosis of the liver, and hepatocellular carcinoma [2–4], but the pathogenic role of this virus in liver disease is still debated [4, 5]. To date, no pathology has been firmly associated with TTV.

The epidemiology of TTV infection is also poorly understood. Large epidemiology studies are limited by the lack of a reliable serological assay for TTV; the only diagnostic tool to indicate an ongoing infection is detection of viral DNA by use of PCR. The carriage rates of circulating viral DNA that have been reported for the general population vary widely: ~1%–10% of the population in the United Kingdom [6, 7], 5% in France [8], 7%–36% in Thailand [3, 9], 10%–42% in United States [10, 11], 37% in New Zealand [12] and 12%–92% in Japan [13, 14]. Although these observations could be a result of uneven geographic distribution of the infection, evidence also indicates that the PCR methods that have been used vary substantially in sensitivity, which may greatly affect the results of prevalence studies [12, 14, 15].

Although TTV is considered to be transmitted by means of blood transfusion [1, 6, 13], its prevalence in the general population is much higher than the prevalence of other transfusion-transmissible viruses [11–14]. The existence of nonparenteral routes of transmission is supported by the detection of TTV in specimens other than blood, including specimens of feces [16, 17], saliva [17], and breast milk [18]. In this study, we examined the presence of TTV in the cervices of pregnant women to examine the possibility of perinatal and sexual transmission.

Cervical samples were obtained from 100 consecutive pregnant Chinese women aged 18–40 years (mean ± SD, 28.8 ± 4.8) who were at various stages of gestation (range, 6–35 weeks; mean ± SD, 17.8 ± 5.9). None of the women had ever received blood products.

TTV DNA was detected by use of 2 types of PCR. The first type, NG (the original clone N22–based genomic sequences) heminested PCR with use of primers NG059, NG061 and NG063 [13], has been frequently used for initial studies of TTV infection. Although it has subsequently been shown to have suboptimal sensitivity [14, 18], it was used because it targets a region of the gene ORF1 where sequence variability allows the genotype to be identified. Briefly, the first round of PCR was performed with primers NG059 (5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) and NG063 (5′-CTG GCA TTT TAC CAT TTC CAA AGT T-3′) for 30 cycles (94°C for 1 min, 58°C for 1 min and 72°C for 1 min), plus an additional 8-min extension at 72°C. For the second round of PCR, primers NG061 (5′-GGC AAC ATG YTR TGG ATA GAC TGG-3′; Y = T/C and R = A/G) and NG063 were used, and samples were run for 30 cycles under the same conditions.

The other type of PCR, nested PCR, targets a relatively conserved region (nt3087–3392, AB008394) within a presumed noncoding region, and has been shown to have sensitivity superior to other commonly used primers [15]. For this assay, primers 5′-GTG GGA CTT TCA CTT GTC GGT GTC-3′ and 5′-GAC AAA TGG CAA GAA GAT AAA GGC C-3′ were used for the first round of amplification, and for the second round, primers 5′-AGG TCA CTA AGC ACT CCG AGC G-3′ and 5′-GCG AAG TCT GGC CCC ACT CAC-3′ were used. Both rounds were run for 30 cycles under the same conditions as were used for NG heminested PCR, but with annealing at 55°C. Samples that had tested positive for TTV DNA by use of NG heminested PCR were further subjected to restriction frag-

Received 20 April 2000; revised 22 September 2000; electronically published 9 April 2001.

Written consent was obtained from all patients included in this study, and the study protocol was approved by the Clinical Research Ethics Committee of The Chinese University of Hong Kong.

Reprints or correspondence: Dr. Paul K. S. Chan, Dept. of Microbiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China (paulkschan@cuhk.edu.hk).

Clinical Infectious Diseases 2001;32:1376–7

1058-4838/2001/3209-0019$03.00

ment–length analysis with use of the restriction enzymes *Nde* I, *Pst*I, *Nla*III and *Mse*I for genotype identification, according to a protocol described elsewhere [19].

Of the 100 pregnant women, 66 (66%) had TTV DNA detected in their cervical samples by use of nested PCR. There was no significant difference in age and gestational stage distribution between the TTV-positive and TTV-negative groups (mean age, 29.4 vs. 27.9 years, $P = .169$, by use of Student's t test; mean duration of gestation, 29.4 vs. 28.8 weeks, $P = .33$, by use of Student's t test). Fifteen samples (15%) tested positive for TTV DNA by use of NG heminested PCR; these samples also tested positive by use of nested PCR. Five of these 15 samples were genotype 1, 2 were genotype 2, 1 was genotype 3, 2 were genotype 4 and the remaining 5 samples were genotype 5. Neither genotype 6 nor coinfection with multiple genotypes was identified.

The observed difference in the rate of positive results for the 2 types of PCR accords with the findings of previous studies, which showed that rates of positive results for NG heminested PCR were about 4–6 fold lower than rates for primer sets that target other parts of the genome [14, 15, 18]. When the analytic sensitivity of the 2 types of PCR was compared, nested PCR was 100-fold more sensitive than was NG heminested PCR. This difference might be accounted for by sequence variation within NG059/061/063 primer binding sites but also by the amplification efficiency of the primer itself, because NG063 has a high AT content (64%) and also a T at the 3′ end. It is generally accepted that PCR primers with high AT content and the presence of A or T at the 3′ end are associated with poor amplification efficiency. Our observation, like those published elsewhere [14, 18], suggests that studies that have used the NG059/061/063 primers might have underestimated the prevalence of TTV.

For all the women in our study, a portion of the cervical sample was tested by means of the Papanicolaou smear. No smears revealed any significant pathological findings, with the exception of 2 smears that revealed atypical cells of undetermined significance. Although TTV DNA was detected in both of these samples, the overall picture suggests that TTV infection of the cervix is not associated with any observable cytologic pathology.

Our results show that the uterine cervix is susceptible to infection by most genotypes of TTV. Moreover, asymptomatic TTV infection of the cervix is common in pregnant women, and the rate of prevalence does not vary significantly with gestational stage. Therefore, vaginally delivered newborns are at risk for exposure to TTV and perinatal transmission of the virus. Our data also indicate that, in addition to blood, feces, saliva, and breast milk, the uterine cervix is a body site where the carriage of TTV is common; there is, therefore, a potential for sexual transmission of this novel virus.

References

1. Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun **1997**; 241:92–7.
2. Hohne M, Berg T, Muller AR, Schreier E. Detection of sequences of TT virus, a novel DNA virus, in German patients. J Gen Virol **1998**; 79:2761–4.
3. Tanaka H, Okamoto H, Luengrojanakul P, et al. Infection with an unenveloped DNA virus (TTV) associated with posttransfusion non–A to G hepatitis in hepatitis patients and healthy blood donors in Thailand. J Med Virol **1998**; 56:234–8.
4. Tagger A, Donato F, Ribero ML, et al. A case-control study on a novel DNA virus (TT virus) infection and hepatocellular carcinoma. Hepatology **1999**; 30:294–9.
5. Maggi F, Fornai C, Morrica A, et al. High prevalence of TT virus viremia in Italian patients, regardless of age, clinical diagnosis, and previous interferon treatment. J Infect Dis **1999**; 180:838–42.
6. Simmonds P, Davidson F, Lycett C, et al. Detection of a novel DNA virus (TTV) in blood donors and blood products. Lancet **1998**; 352: 191–5.
7. Naoumov NV, Petrova EP, Thomas MG, Williams R. Presence of a newly described human DNA virus (TTV) in patients with liver disease. Lancet **1998**; 352:195–7.
8. Biagini P, Gallian P, Cantaloube JF, De Micco P, De Lamballerie X. Presence of TT virus in French blood donors and intravenous drug users. J Hepatol **1998**; 29:684–5.
9. Poovorawan Y, Theamboonlers A, Jantaradsamee P, Kaew-In N, Hirsch P, Tangkitvanich P. Hepatitis TT virus infection in high-risk groups. Infection **1998**; 26:355–8.
10. Desai SM, Muerhoff AS, Leary TP, et al. Prevalence of TT virus infection in US blood donors and populations at risk for acquiring parentally transmitted viruses. J Infect Dis **1999**; 179:1242–4.
11. Handa A, Dickstein B, Young NS, Brown KE. Prevalence of the newly described human circovirus, TTV, in United States blood donors. Transfusion **2000**; 40:245–51.
12. Werno AM, Wang Z, Schroeder BA, Woodfield G, Croxson MC. Prevalence and phylogenetic characterisation of TT-virus in the blood donor population of Auckland, New Zealand. J Med Virol **2000**; 62:109–14.
13. Okamoto H, Nishizawa T, Kato N, et al. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol Res **1998**; 10:1–16.
14. Takahashi K, Hoshino H, Ohta Y, Yoshida N, Mishiro S. Very high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. Hepatol Res **1998**; 12:233–9.
15. Leary TP, Erker JC, Chalmers ML, Desai SM, Mushahwar IK. Improved detection systems for TT virus reveal high prevalence in humans, nonhuman primates and farm animals. J Gen Virol **1999**; 80:2115–20.
16. Okamoto H, Akahane Y, Ukita M, et al. Fecal excretion of a nonenveloped DNA virus (TTV) associated with posttransfusion non-A–G hepatitis. J Med Virol **1998**; 56:128–32.
17. Ross RS, Viazov S, Runde V, Schaefer UW, Roggendorf M. Detection of TT virus DNA in specimens other than blood. J Clin Virol **1999**; 13:181–4.
18. Toyoda H, Naruse M, Yokozaki S, et al. Prevalence of infection with TT virus (TTV), a novel DNA virus, in healthy Japanese subjects, newborn infants, cord blood and breast milk. J Infect **1999**; 38:198–9.
19. Tanaka Y, Mizokami M, Orito E, et al. New genotypes of TT virus (TTV) and a genotyping assay based on restriction fragment length polymorphism. FEBS Lett **1998**; 437:201–6.