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Transfusion transmitted virus infection in general populations and patients with various liver diseases in south China

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INTRODUCTION

Although several specific detecting methods had been applied to determine the hepatitis virus, there was a lot of cryptogenic hepatitis without any known hepatitis infectious marker[1]. The prevalence of hepatitis G virus (HGV) (also known as GB-C virus) infection has been reported to be 5% -13% in patients with non-A-E hepatitis and cirrhosis, however, there is little evidence suggesting that HGV causes hepatitis in human[2-6]. Although cryptogenic liver diseases are almost certainly related to a variety of etiologies, one or more as-yet-unidentified infectious agents are likely to account for a proportion of these cases.

In December 1997, a novel DNA virus was reported by Nishizawa *et al*[7] to be associated with elevated aminotransferase levels in patients with post-transfusion hepatitis of unknown etiology (non-A-G hepatitis). This virus was designated transfusion transmitted virus (TTV). Then, Luo *et al*[8] and Wei *et al*[9] also detected TTV in the sera of patients from an outbreak of cryptogenic hepatitis in south China. And TTV was also detected in patients with post-transfusion hepatitis in China[10].In subsequent analyses, TTV is an un-enveloped single-stranded DNA virus for which a sequence of 3800 bases was determined[11]. Evidence of potential hepatotropism of TTV was reported with TTV DNA detected in liver tissue[12]. Histopathological study indicated that the characteristics of liver histology of TTV infected patients are portal inflammation and interlobular bile duct damage[13]. TTV was proposed as the part of causative agent of non-A to G hepatitis. Seroepidemiological studies have shown TTV to have global distribution[12,14,15]. Although the potential association of TTV with cryptogenic hepatitis is intriguing, the pathological and clinical significance of this virus remains to be established. To assess more thoroughly the etiological role of TTV in the causation of hepatitis, we determined the frequency of TTV infections and their relationship to liver disease in several cohorts of liver diseases and rural population.

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PATIENTS AND METHODS

Rural population

Nighty males and 89 females aged from 1 to 73 years were from a natural village with total population of 190 in southeast of Yunnan Province. Among them, 90 persons were of nationality of Han, others of Yi.

Patients with cryptogenic hepatitis

Forty-four patients with cryptogenic hepatitis were admitted in hospital between January 1993 and May 1999, with negative assays for sera marker of hepatitis virus A-E, and also negative assays for anti-nuclear antibody, anti-smooth muscle antibody, EB virus antibody, CMV antibody and anti-mitochon drial antibody. Part of patients were confirmed with liver biopsy suggestive of acute hepatitis.

Patients with HBV related chronic liver diseases

The prevalence of TTV infection was also determined in five cohorts of HBV related chronic liver disease: ①HBsAg asymptomatic carrier (AsC) (n=52); ② Chronic hepatitis B (CHB) (n = 46); ③ Chronic hepatic failure (n=40); ④ Active liver cirrhosis (n=39); ⑤ Hepatocyte carcinoma (HCC) (n=21). The diagnosis accorded with diagnostic criterion of viral hepatitis in the Fifth Science Meeting of Infectious Disease and Verminosis (Beijing, 1995)[16].

Nested PCR for the detection of TTV DNA

Evidence for TTV infection was determined by detection of TTV DNA by nested PCR. Nucleic acids were extracted from 100μL serum. TTV DNA was determined by PCR with nested primers described by Okamoto *et al*[11] that sensitively detect TTV DNA, irrespective of different

genotypes, as well as by Ampli-Taq- DNA Polymerase. In brief, the first round of PCR was performed with RD038 primer (sense: 5'-TGA CTG TGC TAA AGC CTC TA-3') and NG059 (antisense: 5'-ACA GAC AGA GGA GAA GGC AAC ATG-3') for 35 cycles (94℃, 45 seconds; 54℃, 45 seconds; 72℃, 60 seconds [additional 7 minutes for the last cycle]), and the second-round PCR was performed with NG061 (sense: 5'-GGC AAC ATG TTA TGG ATA GAC TGG-3') and NG063 (antisense: 5'-GAC CGT AAA ATG GTA AAG GTT TCA-3') for 35 cycles with the same conditions. The size of the second-round PCR was 271bp. The amplicons were electrophoresed in 2% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light. All assays were performed in an amplicon-free work area. Positive and negative results were confirmed with repeated assays.

Direct sequencing of the amplicons

Direct sequencing of the amplicons was carried out by Cybersyn.

Statistical analysis

Prevalence of TTV infection (as measured by TTV DNA detectable in serum by PCR) in rural population and several cohorts of liver disease was determined. Data analysis was carried out using χ^2 test. And the liver function test of chronic hepatitis B was analysed using *t* test.

RESULTS

Sequencing of the amplicons (Figure 1)

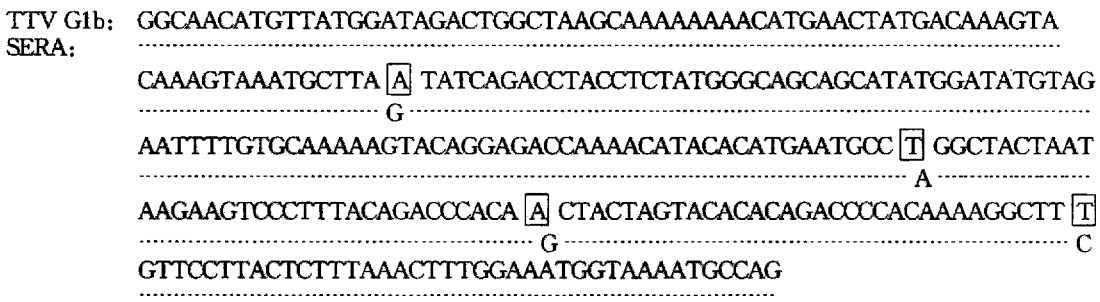

Figure 1 Comparison of nucleic acids sequence bet ween TTV G1b and amplicons and nucleic acids sequence nt1935-2205 of TTV G1b, sera amplicons had a homogeneity of 98.5%, suggesting the presence of TTV DNA in sera.

Prevalence of TTV infection (Table 1)

Table 1 Prevalence of TTV infection among study cohorts

Groups	TTV positive rate (*n*)	TTV negative rate (*n*)	Total
Rural population	10.61(19)	89.39(160)	179
Cryptogenic hepatitis[a]	38.63(17)	61.37(27)	44
HBsAg asymptomatic carrier	9.62 (5)	90.38(47)	52
Chronic hepatitis B[b]	15.22 (7)	84.78(39)	46
HBV related active liver cirrhosis[c]	22.5 (9)	77.5(31)	40
HBV related hepatic failure[d]	23.08 (9)	76.92(30)	39
Hepatocyte carcinoma[e]	9.52 (2)	90.48(19)	21
Total	16.15(68)	83.85(353)	421

[a]χ^2 = 20.486, *P*<0.005 *vs* rural population; [b]χ^2 = 0.713, *P* > 0.25 *vs* HBsAg asymptomatic carrier; [c]χ^2=0.749, *P*>0.25 *vs* chronic hepatitis B; [d]χ^2=0.853, *P*>0.25 *vs* chronic hepatitis B; [e]χ^2=0.000, *P*>0.9 *vs* HBsAg asymptomatic carrier.

Rural population

Ninteen of 179 (10.61%) unselected healthy peoples were detected TTV DNA positive. The prevalence of TTV infection was inde pendent of sex, age and nationality (Table 2).

Table 2 The prevalence of TTV infection in a natural village in Yunnan Province, regarding of sex, nationality and age

Result	Sex[a]			Nationality[b]			Age[c]			
	Male	Female	Total	Han	Yi	Total	<14	14-55	>55	Total
TTV positive	7	12	19	8	11	19	5	13	1	19
TTV negative	83	77	160	82	78	160	47	102	11	160
Total	90	89	179	90	89	179	52	115	12	179
Prevalence(%)	7.8	13.5	10.6	8.9	12.4	10.6	9.6	11.3	8.3	10.6

[a]χ^2=1.535, *P*>0.2; [b]χ^2=0.568, *P*>0.4; [c]χ^2=0.178, *P*>0.9.

Patients with cryptogenic hepatitis

The prevalence of TTV infection in patients with cryptogenic hepatitis was 38.63% (17 of 44). Two of the TTV-infected patients with fulminant hepatic failure, while the others with mild hepatitis.

Patients with HBV related chronic liver disease

The prevalence of TTV infection of AsC, CHB, ALC, CHF and HCC was 9.62% (5/4 7), 15.22% (7/39), 22.5% (9/31), 23.08% (9/30) and 9.52% (2/19), respectively. It seemed that the state of illness was related with the co-infection of TTV. However, there was no statistical difference between the prevalences (Table 1).

Effect on liver function test of TTV co-infection

Whileco-infected with TTV, the state of illness did not exacerbate in patients with CHB (Table 3). In patients with active liver cirrhosis and chronic hepatic failure, there were no significant differences in results of laboratory tests in patients with and without TTV DNA (P>0.2) whereas the co-infection of TTV increased the mortality of patients with hepatic failure (P=0.038).

Table 3 Effect on liver function test of TTV co-infection in patients with chronic hepatitis B

Liver function test	TTV negative	TTV positive	*t* value	*P* value
ALT(U/L)	347.0±286.5	282.3±230.2	0.523	>0.5
AST(U/L)	199.9±171.8	140.8±105.3	0.742	>0.2
Protein A/G	1.24±0.28	1.26±0.12	0.140	>0.5
TBil(μmol/L)	34.1±30.6	22.8±9.3	0.883	>0.2
Prothrombin (s)	17.7±5.5	15.6±1.53	0.783	>0.2

DISCUSSION

The recent discovery in Japan by Nishizawa *et al*[7] of a novel parenterally transmissible, unenveloped, single-stranded DNA virus (TTV) in patients with non-A-G posttransfusion hepatitis had raised some important questions about TTV as a potential cause of liver disease.

Previous study indicated that TTV infection was common in healthy individuals, blood donors, HBsAg AsC, patients on hemodialysis and patients with various liver diseases, however played a minimal role on liver disease[17-26]. Another paper suggested TTV may cause chronic hepatitis in a limited number of patients, but remains dormant most of the time[27]. In our study, we found 10.61% of healthy individuals with normal liver function test were infected with TTV, and the prevalence was independent of sex, age and nationality. The result indicated TTV infection was common in healthy individuals. However, frequency of TTV infection in patients with cryptogenic hepatitis was significantly higher. As evidence of potential hepatotropism of TTV had been reported with TTV-DNA titers shown to be 10 to 100 folds greater in liver tissue than in serum[11,12], TTV would account for part of the reason for patients with cryptogenic hepatitis. As we know that there are many HBsAg AsC, whereas HBV causes many hepatitises. This study suggested that the majority of individuals with TTV could be asymptomatic carriers, with only a small proportion of carriers actually developing hepatitis.

Generally, co-infection of hepatitis virus would lead to exacerbation of hepatitis. While co-infected with hepatitis A and B virus, patients encountered exacerbation of illness, with more severe abnormal results of laboratory tests and higher mortality[28]. In histopathological evaluation, co-infection of hepatitis virus had no significant difference in development of liver cirrhos is[29]. Previous study indicated that superinfection of TTV does not exert deleterious effects on the liver disease induced by HCV. Triple infection, HCV and TTV plus HBV or HGV, did not cause severe liver disease[27]. In current study, prevalence of TTV infection in HBsAg AsC and patients with chronic hepatitis B, HBV related liver cirrhosis and chronic hepatic failure was 9.62%, 15.22%, 22.5% and 23.08% respectively. There was no significant difference among prevalence of TTV infection, suggesting the minor effect on liver disease of co-infection of TTV. The comparison of levels of ALT, AST, total bilirubin, A/G of serum protein and prothrombin time between TT virus-positive and-negative patients did not show any differences, as accorded with another report[30]. However, TTV co-infection increased the mortality of patients with hepatic failure.

Most cases of chronic hepatitis, cirrhosis, and HCC in developed countries are caused by HBV or HCV infections and heavy alcohol intake. A relatively small proportion of liver diseases is of unknown etiology. The recently discovered HGV seems to have no actual role in causing acute or chronic liver disease[31], whereas the role of the more recently discovered TTV is still to be defined. To inquire the relationship between TTV infection and HCC, we investigated the prevalence of TTV infection in HBV infected patients with HCC, and compared with HBsAg AsC. The result indicated similar prevalence in both cohorts (9.52% *vs* 9.62%, P>0.9), which did not support the hypothesis of an association between TTV infection and HCC, and accorded with previous studies[32,33]. Another report indicated that TTV was not specific for HBV-negative and HCV-negative patients with HCC. For all TTV-positive patients, the TTV genome was not integrated into host hepatocyte DNA[34] . In conclusion, TTV was common in general population and several cohorts of liver disease. Though the majority of individuals with TTV could be asymptomatic carriers, TTV would account for part of cryptogenic hepatitis. As TTV

co-infection did not affect the state of HBV infection, further study on pathogenic effect on liver disease of TTV infection should be continued.

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