RESEARCH NOTE

TORQUETENOVIRUS INFECTION AMONG NORTHEASTERN THAI BLOOD DONORS

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Abstract. Semi-nested polymerase chain reaction technique was used to detect Torquetenovirus (TTV) DNA in 234 healthy blood donors in northeast Thailand. The incidence of TTV was 28% in 101 healthy blood donors negative for HBsAg and anti-HCV antibody, 25% in 71 HBsAg carriers and 29% among 62 with anti-HCV antibody. No association of TTV infection was found with gender, age, and HBV or HCV infection.

INTRODUCTION

Torquetenovirus (TTV) or transfusion transmitted virus, formerly known as TT virus, is a member of the genus *Anellovirus*, which is an unenveloped, negative single-stranded circular DNA virus with 3,739 nucleotides (Okamoto *et al*, 1998). TTV was first described in Japan as a DNA virus in the blood of a patient with post-transfusion cryptogenetic hepatitis (Nishizawa *et al*, 1997). This led to the conclusion that the virus is hepatotropic and can be considered as one of causative agents of hepatitis.

Hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV antibody) are used to identify blood donors who may carry or have been exposed to hepatitis virus. When HBsAg or anti-HCV is positive, the person is deferred and cannot be accepted as a blood donor under any circumstances. In Thailand, chronic hepatitis B virus (HBV) and, to a lesser extent, chronic hepatitis C virus (HCV) infections represent the two most common causes of hepatitis potentially proceeding to chronic liver disease (Tangkijvanich *et al*, 1999b). HBV is associated with the development of hepatocellular carcinoma among the Thai population (Tangkijvanich *et al*, 1999a).

Although the association of TTV with liver disease remains unclear, the prevalence of TTV in various populations have been studied. TTV prevalence in healthy blood donors varies from 1.9% in UK (Simmonds *et al*, 1998), 3.15% in New Zealand (Werno *et al*, 2000), 6.2% in Iceland (Love *et al*, 2000), 6.7-7.4% in western India (Arankalle *et al*, 2000), 28% in Egypt (Gad *et al*, 2000, Hassoba *et al*, 2003), 28% in China (He *et al*, 1999), 42.9% in Mongolia (Kato *et al*, 1999) and 46% in Brazil (Devalle *et al*, 2004). TTV prevalence in hepatitis patients varies from 27.1-66.7% in Egypt (Gad *et al*, 2000, Hassoba *et al*, 2003), 32-45% in China (He *et al*, 1999), 54% in Brazil (Devalle *et al*, 2004) and 60.2% in Mongolia (Kato *et al*, 1999). In

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Thailand, the prevalence of TTV was 20% in chronic hepatitis patients (Tangkijvanich *et al*, 1999c), and 17% and 48% in blood donors younger than and older than 20, respectively (Udomsakdi-Auewarakul *et al*, 2000). Infections of HBV, HCV (Barusrux *et al*, 1997) and HGV (Barusrux *et al*, 2006) but not HIV (Urwijitaroon *et al*, 1996) and HTLV-I (Barusrux *et al*, 1995; Urwijitaroon *et al*, 1997) are very common in the northeastern part of Thailand but there has been no report of the prevalence of TTV infection in this region. In this study, we used semi-nested PCR to examine the incidence of TTV in healthy blood donors in northeastern Thailand and evaluated the association between TTV viremia and HBV and HCV infection.

MATERIALS AND METHODS

Serum samples and study population

Serum samples were collected from apparently healthy blood donors who donated blood during June 1998 to December 1998 at Blood Center, Faculty of Medicine, Khon Kaen University, which is situated in Khon Kaen Province, central part of northeast region of Thailand. They included 101 blood donors who were negative for HBsAg and anti-HCV antibody (group I), 71 positive for HBsAg (group II) and 62 positive for anti-HCV antibody (group III).

DNA preparation

DNA was extracted from 100 μl of serum using Smitest Ex-R&D kit (Somitomo Metal Industries, Japan). Extracted DNA was dissolved in 40 μl of TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and 10 μl was used for TTV DNA detection.

Detection of TTV DNA

TTV DNA was amplified by semi-nested PCR with TTV-specific primers (Okamoto *et al*, 1998). First round PCR was performed with two primers, NG059 (sense : 5′-ACAGACAG AGGAGAAGGCAACATG -3′) and NG063 (antisense : 5′-CTGGCATTTTACCATTTCC AAAGTT-3′). Primers for the second round PCR were NG061 (sense : 5′-GGCAACATGY TRTGG ATAGACTGG -3′ where Y = C or T, and R = A or G) and HG063. Ten μl of each DNA sample was used for amplification. The first round amplification was carried out for 35 cycles at 94ºC for 30 seconds, 58ºC for 30 seconds and 72ºC for 45 seconds, followed by 7 minutes at 72ºC after the last cycle. Five μl of the first round PCR product was used for the second round amplification, which was performed for 25 cycles at 94ºC for 30 seconds, 58ºC for 30 seconds and 72ºC for 30 seconds with an additional extension step for 7 minutes at 72ºC. The amplification product of the first round PCR was 286 bp and that of the second round PCR was 271 bp. Positive and negative controls were included in each PCR assay. Amplicons were analyzed by electrophoresis in 2% agarose gel with ethidium bromide staining and observed under ultraviolet light.

Statistical analysis

The Yates corrected χ^2 test and Fisher's exact test were used to analyze the data. P-value of less than 0.05 is considered statistically significant.

RESULTS

The incidence of TTV was 28% (28 cases) in group I, 25% (18 cases) in group II, and 29% (18 cases) in group III (Table1). There are no statistical differences among the mean age of these three groups, although females were uncommon in anti-HCV positive group. There is no statistical difference in the distribution of males and females among the three groups. Analysis of TTV infection among different age distribution (Table 2) and history of tattoo and/or intravenous drug users among the HCV-infected blood donors (Table 3) also demonstrated no statistically significant difference.

DISCUSSION

This study indicates that TTV infection is

Table 1
TTV incidence and characteristics of northeastern Thai blood donors.

Feature	Group I	Group II	Group III
	Negative HBsAg/anti-HCV	Positive HBsAg	Positive anti-HCV
Number tested	101	71	62
Mean age ± SD	28.8 ± 9.8	26.6 ± 11.7	33.1 ± 7.7
Female : Male	50 : 51	29 : 42	7 : 55
Female : TTV positive (%)	15 (30%)	5 (17%)	2 (29%)
Male : TTV positive (%)	13 (25%)	13 (31%)	16 (29%)
Total : TTV positive	28 (28%)	18 (25%)	18 (29%)

Table 2
Age distribution of TTV positive blood donors.

Age range (year)	Number	Positive	
		Number	%
16-25	112	29	26
26-35	48	11	23
36-45	61	17	28
46-55	11	6	54
56-65	2	1	50
Total	234	64	27

Table 3
TTV infection and history of tattoo and /or intravenous drug users among the HCV infected blood donors.

History of tattoo and /or IVDU	Negative for TTV	Positivefor TTV
Yes	27	8
No	17	10

common in northeast Thailand, but there is no statistically significant difference of TTV incidence among different age groups blood donors ($p>0.05$). The incidence of TTV infection among the donors with history of tattooing and/or IVDU also showed no significant difference. Thus, parenteral transmission was unlikely to be the main route of TTV infection. These findings are in contrast with the previous study in 70 patients with various types of chronic hepatitis and 100 healthy subjects from New Delhi, India, where TTV DNA was detected in 26% with chronic HBV hepatitis, 15% with chronic HCV hepatitis, and 12% from healthy control group with normal liver function tests (Chattopadhyay *et al*, 2005). Among Egyptian patients with HCC, TTV infection is not associated with HCV, HBV, NBNC-HCC, history of schistosomiasis or blood transfusion (Hassoba *et al*, 2003). In eastern Anatolia, Turkey, there is no significant difference in the positive cases for TTV and HGV according to duration of illness or mean duration of institutionalization (KalKan *et al*, 2005). Although, TTV appears to be highly prevalent worldwide, its etiology and contribution to liver disease need further clarification.

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