High Prevalence of Transfusion-Transmitted Virus Infection in Patients with Chronic Liver Diseases in an Endemic Area of Hepatitis B and C Virus

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Key Words

Transfusion-transmitted virus · Prevalence · Chronic liver diseases

Abstract

Objective: To determine the prevalence and clinical impact of transfusion-transmitted virus (TTV) DNA in patients with chronic liver diseases in the Southeast Anatolia region of Turkey where hepatitis B and C viral infections are endemic. **Subjects and Methods:** Patients diagnosed with chronic liver disease by clinical, biochemical and histologic means were enrolled in the study. Serum samples of 60 patients (19 males, 41 females) with chronic liver diseases, and of 45 healthy volunteer blood donors as a control group were collected. The chronic liver disease group consisted of 11 patients with hepatitis B, 44 with hepatitis C and 5 with chronic liver disease of unknown etiology. Presence of TTV DNA was investigated by the polymerase chain reaction. Using a scoring system histological grading of inflammation and staging of fibrosis were performed only in the chronic hepatitis C group. **Results:** TTV DNA was detected in 47 (78%) patients with chronic liver disease and 5 (11%) volunteers in the control group. The difference was statistically

significant ($p < 0.001$). Ten of the 11 (91%) patients with hepatitis B, 32 of 44 (73%) of those with hepatitis C-related chronic liver disease, and 5 of 5 (100%) of the patients with cryptogenic liver disease were positive for TTV DNA. **Conclusion:** TTV is highly prevalent in patients with chronic liver diseases in Southeast Anatolia, Turkey but no pathogenic effect attributable to TTV infection was detected.

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Introduction

TT virus is a recently discovered DNA virus that is associated with acute posttransfusion hepatitis of unknown etiology. Initially it was named after the initials (T.T.) of the first patient, but it has been renamed transfusion-transmitted virus (TTV). TTV is a human circovirus with nonenveloped, single-stranded circular DNA, with a particle size of 30–50 nm, comprising a genome of 3.8 kb. An important feature of TTV is an extremely high degree of genomic variability [1–4].

Since the first discovery of TTV in late 1997, a number of investigators have studied the prevalence of TTV DNA in different population groups and geographic areas of the

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world. In healthy blood donors, the prevalence range of TTV DNA is 1–8% in the USA, 1.5–27% in Europe, 11–43% in Asia, 29–83% in Africa, and 10–90% in Japan [5–8]. Although recent studies using a more sensitive, new primer set for the virus have shown a very high prevalence of TTV DNA positivity (up to 93%) [9–11], its prevalence varies widely according to geographic regions. Hence, we decided to investigate the prevalence of TTV in patients with chronic liver diseases and healthy people in the Southeast Anatolia region of Turkey where viral hepatitis B and C are endemic.

Subjects and Methods

Sixty patients with chronic liver diseases and 45 volunteers were involved in the study. The patients were chosen from the patients who attended the Gastroenterology Clinic of Gaziantep University, Medical Faculty between May and November 2000 and the volunteers were first-time blood donors at the blood bank of the university. The study protocol was approved by the local ethics committee and all the patients gave informed consent. According to clinical, serological and histopathological features, the patients with chronic liver disease consisted of 11 patients with hepatitis B and 44 patients with hepatitis C virus (HCV) infection, and 5 patients with hepatitis of unknown etiology (cryptogenic).

The mean age of the chronic hepatitis B group (4 males and 7 females) was 40.3 ± 16.4 years. Only 1 patient in this group had transfusion as a risk factor. The diagnosis was based on the presence of elevation of alanine aminotransferase (ALT, 46.4 ± 26.4 IU/l), aspartate aminotransferase (AST, 47.1 ± 20.6 IU/l), a positive test for HBs antigen for at least 6 months, and histopathologically proven chronic inflammation of the liver.

The mean age of the chronic hepatitis C group (13 males and 31 females) was 43.8 ± 14.5 years. Eight of them had a history of transfusion. Inclusion criteria to this group were elevated ALT (71.6 ± 48.2 IU/l), AST (70.4 ± 56.2 IU/l), a positive test for anti-HCV antibodies and for HCV RNA, and histologically proven chronic inflammation of the liver. Histologic grading of inflammation and staging of fibrosis was performed in the chronic hepatitis C group by using a modified scoring system [12]. Hepatic inflammation was classified as follows: none = 0; minimal = 1; mild = 2; moderate = 3, and severe = 4. Stage of fibrosis was assessed by using a five-grade scale: none = 0; mild (portal expansion) = 1; moderate (portoportal septa) = 2; severe (bridging and septal fibrosis) = 3, and cirrhosis = 4.

The mean age of the cryptogenic group of 5 patients (2 males and 3 females) was 46.4 ± 15.8 years; ALT and AST levels were 56.8 ± 32.2 and 62.4 ± 28.8 IU/l, respectively. All viral, metabolic and autoimmune markers were negative. None of the patients drank alcohol regularly and/or took hepatotoxic drug(s).

The control group consisted of 45 healthy volunteers (30 males and 15 females, mean age 41.2 ± 12.5 years) with normal liver enzyme levels and negative tests for HBs antigen and anti-HCV. All denied risk practices for parenteral transmission of infectious agents.

Blood was obtained from both the study and control subjects. Each serum sample (100 µl) was pretreated with pronase E and its

Table 1. Clinical and hepatic histological characteristics of patients with chronic hepatitis C according to the presence and absence of TTV infection

	TTV DNA (+) hepatitis C patients	TTV DNA (-) hepatitis C patients	p
Patients	32	12	
Sex (M/F)	6/26	5/7	NS
Mean age, years	53.1	54.2	NS
Transfusion history	5 (15%)	3 (25%)	NS
AST (mean $\pm$ SEM)	62.4 ± 41.6	78.3 ± 57.9	NS
ALT (mean $\pm$ SEM)	65.3 ± 44.3	77.4 ± 60.4	NS
<i>Stages of histopathologic findings</i>			
Inflammation			NS
1	5 (15.6%)	2 (16.7%)	
2	12 (37.5%)	5 (41.6%)	
3	10 (31.3%)	3 (25%)	
4	5 (15.6%)	2 (16.7%)	
Fibrosis			NS
1	5 (15.6%)	1 (8.3%)	
2	8 (25%)	4 (33.3%)	
3	8 (25%)	5 (41.7%)	
4	11 (34.4%)	2 (16.7%)	

buffer at 40°C and 60 min, and then nucleic acids were extracted with alkali-phenol and chloroform. Extracted nucleic acids were dissolved in 20 µl DNase and RNase in free deionized distilled water and then subjected to PCR as described previously [9]. Briefly, the thermocycler was programmed first to preheat at 95°C for 10 min to denature samples and then samples (10 µl of extracted DNA was mixed with 2.0 mM MgCl₂ 30 pmol each primer, sense and antisense, 2 U Taq polymerase, 10 mM dNTP) were subjected to 45 cycles consisting of 94°C for 20 s, 60°C for 20 s, and 72°C for 30 s with an MJ Research thermal cycler. Multiple positive and negative controls were run in each PCR assay. PCR products (199 bp in length) were analyzed in a 2% agarose gel electrophoresis with ethidium bromide staining, and photographed under UV light. The sequences of the TTV-specific primers were T801 (5' gct acg tca cta acc acg tg 3', sense primer, nucleotides 6–25) and T935 (5' ctb cgg tgt gta aac tca cc 3', antisense primer, nucleotides 204–185; b = g, c or t) as designated by Takahashi et al. [9].

Statistical comparison of the groups was made by χ^2 test or Fisher's exact test for categorical variables and by the Student's t test or Mann-Whitney U test when appropriate for quantitative values. A p value < 0.05 was defined as statistically significant.

Results

TTV DNA was detected in 47 of 60 (78%) patients with hepatitis and 5 of 45 (11%) healthy blood donors. The difference between the groups was statistically significant.

cant ($p < 0.001$). The frequency rates of TTV were also significantly high in patients with hepatitis of unknown origin (5 of 5, 100%), hepatitis B infection (10 of 11 patients, 91%) and hepatitis C infection (32 of 44, 73%) as compared to the control group (11%, $p < 0.001$ in all subgroups).

Since the number of patients with hepatitis B or hepatitis of unknown origin was limited, comparison between TTV (+) and TTV (–) was done only for hepatitis C. TTV-infected and noninfected patients with chronic hepatitis C are given in table 1. The results did not reveal any significant differences concerning clinical and laboratory features. No impact of TTV infection was seen on liver histopathologic findings such as grade of inflammation ($p = 0.98$) or stage of fibrosis ($p = 0.51$) in the patients with chronic hepatitis C.

Discussion

Based on the high prevalence of TTV infection in Japanese patients [2] it was initially suggested that TTV could be associated with posttransfusion hepatitis of unknown etiology and other cryptogenic liver diseases. However, high prevalence of the virus in the healthy population [5–8] and the lack of a clear association of TTV infection with a particular group of liver disease in subsequent studies strongly suggested that TTV does not cause hepatic inflammation [13–15]. In this study, we used the new primer set defined by Takahashi et al. [9] and found that only 11% of healthy subjects were TTV-positive, thereby indicating that TTV is not prevalent in the general population of Southeast Anatolia, Turkey.

The role of TTV in chronic hepatitis of unknown etiology has been studied extensively in recent years [2, 16–20]. The prevalence of TTV DNA in patients with cryptogenic chronic liver disease did not differ from that of the general population in Japan [16], Spain [17], Thailand [18] and the USA [19, 20]. The findings of these studies would indicate a lack of association between TTV DNA infection in patients with liver disease of unknown etiology and healthy blood donors.

A high prevalence of TTV (78%) was found in patients with chronic hepatitis B and C infections. However other studies reported lower rates of 20–37% and 5–27% for hepatitis B and C infections, respectively [13, 17, 18, 21, 22]. The high prevalence of hepatitis B (91%) and C (73%) in our region could have contributed to the high prevalence of TTV probably through common transmission routes. Hepatitis B and C infections are very common

within same family members, and therefore mother-to-infant transmission could be a risk factor in our region. An other important route of transmission could be the use of the same needles in infants during previous vaccination programs.

No difference between TTV-positive and -negative chronic hepatitis C patients was noticed in this study. Liver histologic findings such as stage of inflammation and grade of fibrosis did not differ in patients with TTV-positive or -negative chronic hepatitis C infection, thereby indicating minimal or no impact of TTV on hepatic pathology in this study (table 1), a finding consistent with previous reports [22–24]. Due to its wide distribution of TTV and its weak association with disease state, it has been suggested that TTV may represent a part of the human microflora [25].

It is important to point out that in this study in evaluation the prevalence of TTV, we detected only persons with viremia; there could have been patients with active liver infections that were negative on plasma PCR or patients with past exposure as evidenced by serological assays. Newly defined IgM class antibodies may be important in further evaluation of this group of patients [26].

Conclusion

The prevalence of TTV in Southeast Anatolia, Turkey is very high in patients with chronic liver disease, but association with disease state is not clear. Further studies especially with new serologic assays to diagnose acute and past infection are needed.

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