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• CLINICAL RESEARCH •

Coinfection of TT virus and response to interferon therapy in patients with chronic hepatitis B or C

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Abstract

AIM: **To investigate the serum positive percentage of TT virus (TTV) in patients with chronic hepatitis B or C and the response of the coinfected TTV to interferon (IFN) during IFN therapy for chronic hepatitis B and C.**

METHODS: **We retrospectively studied the serum samples of 70 patients with chronic hepatitis who had received IFN-alfa therapy from January 1997 to June 2000, which included 40 cases of hepatitis B and 30 hepatitis C. All the patients had been followed up for at least 6 months after the end of IFN therapy. The serum TTV DNA was detected using the polymerase chain reaction (PCR) before and every month during the course of IFN treatment.**

RESULTS: **TTV infection was detected in 15% (6/40) of the chronic hepatitis B group and 30% (9/30) of the chronic hepatitis C group. Loss of serum TTV DNA during IFN therapy occurred in 3 of 6 patients (50%) and 6 of 9 (67%) of hepatitis B and C groups, respectively. Seronegativity of TTV was found all during the first month of IFN therapy in the 9 patients. There was no correlation between the seroconversion of TTV and the biochemical changes of the patients.**

CONCLUSION: **TTV is not infrequently coinfected in patients with chronic hepatitis B and C in Taiwan, and more than half of the TTV infections are IFN-sensitive. However, the loss of serum TTV DNA does not affect the clinical course of the patients with chronic hepatitis B or C.**

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INTRODUCTION

In 1997, a novel DNA virus was isolated from a patient with post-transfusion hepatitis of unknown etiology in Japan, and was designated as TT virus (TTV) after the initials of the index patient[1]. From then on TTV has been studied worldwide. Now we know that TTV genome is non-enveloped, circular, single-stranded DNA and comprises 3,852 bases with a particle size of 30-50nm. These findings suggest that TTV is closely related to the Circoviridae[2,3].

In the original studies from Japan, the agent was found in 34/290 (12%) of healthy donors, compared to 9/19 (47%) of patients with fulminant non-A to G hepatitis and 41/90 (46%) with non-A-G chronic liver disease[2]. In 72 patients with chronic liver disease in the United Kingdom, TTV DNA was demonstrated in 18 cases (25%), compared to 10% of 30 cases of healthy controls[4]. Chronic liver disease caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection is common in Taiwan[5,6] and interferon (IFN) has been used for the treatment of chronic infections. Therefore, we aimed to study the serum positive percentage of TTV infection in such patients who had received IFN therapy and also to see the response of TTV to IFN during the course of the treatment.

MATERIALS AND METHODS

Patients

We retrospectively studied the frozen-stored serum samples from the patients who had received IFN therapy for chronic hepatitis B and C at Cathay General Hospital from January 1997 to June 2000.

For chronic hepatitis B, we only included the cases with both positive HBsAg (Auszyme, Abbott Lab., North Chicago, Il.) and positive HBeAg [HBe (rDNA) EIA, Abbott Lab.]. Hepatitis C was confirmed with positive results for the anti-HCV antibody (Murex anti-HCV, version Ⅲ, Murex Diagnostics Ltd., Dartford, England). All the cases had elevated serum alanine transaminase (ALT) levels for more than 6 months and had had at least three documented occasions of levels higher than twice the upper limit of normal (<35 IU/L), at least 1 month apart, and within 6 months prior to enrollment. All the patients underwent liver biopsy within 1 month before the start of IFN treatment. The diagnosis of chronic liver disease was based on clinical and pathological results. Serum samples taken from the patients were stored at -70℃ until use.

None of our patients was alcoholic, an intravenous drug abuser or homosexual. None had received hepatotoxic drugs, herbal medicine or immuno-suppressive therapy within the 6 months prior to IFN therapy. Patients with metabolic liver diseases including hemochromatosis, Wilson's disease or α-1 anti-trypsin deficiency and autoimmune hepatitis were excluded by clinical and laboratory examinations. None had decompensated liver function (prolonged prothrombin time > 3 seconds, serum total bilirubin > 3.0 mg/dl, or serum albumin <3.0 gm/dl), chronic renal failure, clotting abnormalities, or serious neurological disorders. Those who coinfected with both HBV and HCV were also excluded. Informed consent for the IFN therapy and examinations, including virological assays, was obtained from all the patients.

Laboratory assays

The patients underwent blood biochemical tests every week for the initial 4 weeks and every 2 weeks thereafter during the treatment until 24 weeks. After the end of the treatment, the patients were followed up at 4-week intervals for 12 months.

Serum samples from hepatitis C patients were examined for HCV RNA using reverse transcription-nested polymerase chain reaction (PCR) with primers for the 5'-noncoding region of HCV RNA. Genotyping of HCV RNA was assayed by PCR with type-specific primers[7]. Serum HBV DNA was quantified with the use of a signal amplified solution hybridization antibody capture assay (Hybrid capture system, Digene, Gaithersburg, MD, USA). The presence of serum HCV RNA or HBV DNA was determined before the initiation of IFN therapy, at the end of therapy, and at 24 weeks after the completion of therapy.

Detection of TTV DNA

Serum TTV DNA was determined in specimens before the initiation of IFN therapy and regularly checked every 1 month during the course of the treatment. TTV DNA was examined using the PCR method with nested primers as previously described[8]. Briefly, DNA was extracted from 100 μL of serum using a QIAMP blood kit (QIAGEN Ltd., Crawley, UK) and resuspended in 50 μL of elution buffer. For the first round of PCR, 25 μL of reaction mixture containing 2 μL of the cDNA sample, 1×PCR buffer (10mM tris-HC1 pH 9.0, 50mM KC1, 1.5mM $MgC1_2$, 0.01% gelatin, and 0.1% Triton X-100), 10mM of each dNTP, 100ng of each outer primer T-1 (sense: 5'-ACA GAC AGA GGA GAA GGC AAC ATG-3') and T-2 (anti-sense: 5'-CTA CCT CCT GGC ATT TTA CC-3'), and 1 unit of Taq DNA polymerase was amplified in a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) for 30 cycles. One microliter of the PCR products was re-amplified for another 30 cycles with 100ng of inner primers, T-3 (sense: 5'-GGC AAC ATG TTA TGG ATA GAC TGG-3') and T-4 (anti-sense: CTG GCA TTT TAC CAT TTC CAA AGT T-3'). The amplified products were separated by 3% agarose gel electrophoresis and stained with ethidium bromide.

Interferon therapy

For the patients with chronic hepatitis B, 10 million units (mu) of recombinant interferon alfa-2b (Intron A, Schering-Plough, Co. Kenilworth, NJ, USA) was subcutaneously administered three times weekly for 24 weeks. For those with hepatitis C, 4.5 million units of recombinant alfa-2a (Roferon-A, F. Hoffmann-La Roche Ltd., Basle, Switzerland) was used subcutaneously three times a week for 24 weeks. The response to IFN was classified into two patterns according to the serum ALT level. Patient who had normalized serum ALT level (<35 IU/L) during therapy and remained constant for up to 6 months after the end of therapy was considered to have a biochemical sustained response. Non-sustained response was defined as serum ALT level that could not be normalized either at the end of therapy or during the follow-up period. The virological sustained response was defined as the absence of HBV DNA and HBeAg for hepatitis B, HCV RNA for hepatitis C and TTV DNA for TTV infection at 6 months after the end of therapy.

Statistical analysis

Data were analyzed by Student's *t* test, Chi-squared test with Yates' correction or Fisher's exact test where appropriate. All statistical tests were two-sided. A *P* value of less than 0.05 was considered significant.

RESULTS

The sera of 70 patients were studied, which included 40 patients with chronic hepatitis B and 30 patients with chronic hepatitis C (Table 1). The difference of ages between the two groups was statistically significant ($P<0.001$). In those with chronic hepatitis B, the mean age was 33 years; whereas in the chronic hepatitis C group, it was 41 years. As for the gender distribution and serum ALT levels, there was no statistically significant difference between the two groups.

Serum TTV DNA could be detected in 6 cases (15%) of chronic hepatitis B and 9 cases (30%) of chronic hepatitis C. However, the difference was not statistically significant ($P=0.130$) (Table 1).

During IFN therapy loss of serum TTV DNA was found in 3 of 6 (50%) TTV-positive patients with chronic hepatitis B and 6 of 9 (67%) TTV-positive patients with chronic hepatitis C (Table 2). Because this was a retrospective study and the doses of IFN used for chronic hepatitis B and C were different, we could not compare the rate of TTV disappearance between the two groups. Of interest, disappearance of serum TTV DNA occurred during the first 4 weeks of IFN therapy in all the 9 cases despite the regimen of treatment or the type of chronic viral hepatitis (Table 2). In addition, serum ALT levels did not change when TTV disappeared from the serum. Serum TTV DNA was still examined every 4 weeks after the cessation of IFN therapy in the 9 cases of TTV seroconversion and had continued for 24 weeks. There was no case having the re-emergence of TTV DNA during this follow-up period.

Disappearance of TTV occurred in different genotypes of chronic hepatitis C (1a: 1/1 case, 1b: 3/5 cases, 2a: 1/2 cases, 2b: 1/1 case.). However, the number of cases was small.

In the group of chronic hepatitis B, 10 patients had virological peristent response to IFN therapy, and all the 10 patients also had biochemical peristent response. Whereas, only one patient (1/3 cases) with virological persistent response of TTV DNA had biochemical persistent response (Table 3). If we further exclude the patient with concomitant loss of HBV DNA, HBeAg and TTV DNA, none of the patients (0/2 cases) with TTV virological persistent response had biochemical persistent response.

In the group of chronic hepatitis C, all the 7 patients with virological persistent response of HCV RNA to IFN therapy had biochemical persistent response, and 2 patients (2/6 cases) with virological sustained response of TTV DNA had biochemical persistent response (Table 4). Nevertheless, none of the patients (0/4 cases) of TTV virological sustained response had biochemical persistent response after exclusion of 2 patients of concomitant loss of TTV DNA and HCV RNA.

Table 1 Demographic data and serum positive percentage of TTV in the two groups

Type	Sex (M/F)	Age (yr)	ALT (IU/L)	TTV DNA	
				No.Positive	%
B[a] (*n*=40)	29/11	33±8[c]	133±65	6	15
C[b] (*n*=30)	24/6	41±9	121±60	9	30

[a]Chronic hepatitis B group. [b]Chronic hepatitis C group. [c]$P<0.001$.

Table 2 Loss of serum TTV DNA during IFN therapy in the two groups

	Time of IFN Therapy (weeks)						Total
	4	8	12	16	20	24	
B[a]	3	0	0	0	0	0	3
C[b]	6	0	0	0	0	0	6

Data are presented as case number. [a]Chronic hepatitis B group. [b]Chronic hepatitis C group.

Table 3 Relationship between viral and biochemical responses in the group of chronic hepatitis B

	Virological SR	
	HBV (*n*=10)	TTV (*n*=3)
Biochemical SR (+)	10	1
Biochemical SR (-)	0	2

SR: sustained response, $P=0.038$, by Fisher's exact test

Table 4 Relationship between viral and biochemical responses in the group of chronic hepatitis C

	Virological SR	
	HCV (*n*=7)	TTV (*n*=6)
Biochemical SR (+)	7	2
Biochemical SR (-)	0	4

SR: sustained response,P=0.021, by Fisher's exact test

DISCUSSION

Epidemiologic studies have confirmed that TTV is a parenterally transmitted agent as demonstrated by donor-recipient linkage in transfused patients and by a high prevalence among hemophiliacs and intravenous drug abusers[9-11]. In general, TTV is common in populations at risk of infection with blood-borne viruses[2,12-14]. Many hepatitis viruses share the same modes of transmission, thus multiple viral infections may occur in a given patient[15].

Coinfection of TTV has been observed frequently in patients with chronic hepatitis B or C[4]. Chronic infection of hepatitis B or C virus is common in Taiwan. Thus, we made use of such patients who underwent interferon treatment to study the TT virus. In our series TTV DNA was detected in 15% of chronic hepatitis B and 30% of chronic hepatitis C, which were comparable to the results of Kao *et al* [16], (22% and 37%, respectively) and apparently higher than that (10%) of healthy adults in Taiwan[8]. In a prior study by Naoumov et al, TTV infection was detected in 21% of 33 patients with chronic hepatitis C and 20% of 10 patients with chronic hepatitis B[4]. In Thailand, Tanaka et al, also found that 36% of 59 patients with HBsAg (+) and 36% of 10 patients with HCV RNA (+) had TTV infection[17]. Several other studies also reported that the serum positive rates of TTV DNA in the patients with chronic hepatitis C, and the range was 20-46%[18-20]. The variation might be due to the different primers used for the detection of TTV DNA. These results imply that HBV, HCV, and TTV may share common modes of transmission[16].

The interferons possess antiproliferative, antiviral and immunomodulant properties[21]. Extensive clinical trials have confirmed the efficacy of recombinant interferon-alfa for patients with chronic hepatitis B, C and D[22-26]. However, because the causal role of TTV in liver disease has not been established, there is only a few papers which studied the response of TTV to IFN therapy. Taking advantage of previous research of IFN therapy for chronic hepatitis B and C, we were able to retrospectively study the prevalence of TTV in these patients and to see the response of TTV to IFN treatment. In our study, loss of serum TTV DNA during IFN therapy was noted in 50% (3/6) of chronic hepatitis B and 67% (6/9) of chronic hepatitis C. Regretfully, the comparison of these results was not feasible because this was a retrospective study and the doses of IFN used in two groups were different. Kao et al[16]. had similar results and they found that 41% (17/41) of patients with HCV and TTV coinfection lost serum TTV DNA at 24 weeks after the end of IFN therapy for chronic hepatitis C. Virological sustained response of TTV DNA after IFN therapy was detected to be 40-55% in patients coinfected with chronic hepatitis C according to the recently published reports[18-20, 27]. These findings suggest that TTV was actually vulnerable and responsive to IFN therapy. In addition, all the 9 IFN-responsive cases in our series lost their TTV DNA within the first 4 weeks of IFN therapy. This kind of seroconversion occurred in the same way in both hepatitis B and C groups, but we need more cases to further observe and confirm this phenomenon. With the results above, we know that TTV could be divided into 2 types according to the response to IFN therapy: IFN-sensitive and IFN-resistant. For the IFN-sensitive virus, the 4-week course of IFN therapy was enough to cause the seronegativity of TTV DNA. Moreover, virological sustained response could be achieved in all the 9 IFN-sensitive cases.

TTV was detected in patients with different genotypes of chronic hepatitis C. Loss of serum TTV DNA during IFN therapy occurred in all the genotypes in our study. The conversion rate between each genotype could not be compared because the number of patients was not enough. Nevertheless, the loss of serum TTV DNA during IFN therapy did not seem to be associated with the genotype of HCV.

Investigations of TTV showed considerable diversity among different isolates. The genetic diversity has continued to expand as more and more isolates have been studied[2,4,12]. The different response patterns to IFN therapy must be related to the genetic diversity. Comparison of partial viral DNA nucleotide sequences and phylogenetic analysis done by Chayama *et al*[27] showed that viral strains that had a high identity to the prototype virus were more resistant to IFN than those showing low nucleotide sequence identity. The variants with multiple substitutions in the genomic sequence were more apt to be eliminated by IFN. Further analysis with new genotyping assays will reveal more information in this field.

During the course of IFN treatment, we did not find any correlation between the seroconversion of TTV DNA and the change of serum ALT levels. Although TTV was sensitive to IFN therapy in many subjects, the improvement in ALT levels after IFN therapy was not attributable to the eradication of TTV but rather to that of HCV or HBV. The disappearance of TTV DNA had no effect on the biochemical response to IFN therapy[18,20]. According to such results, TTV may lack pathogenicity or clinical association with liver disease in these patients, which is consistent with the conclusions of many other reports[4,8,28,29].

In summary, the serum positive percentage of TTV in chronic hepatitis B or C in our series was not low. During the IFN therapy for chronic hepatitis B or C, disappearance of coinfected TTV occurred in more than half of the patients. IFN-sensitive TTV usually lost its DNA during the first month of treatment. Genotyping of TTV might further clarify the cause of diverse responses to IFN therapy. The finding that the disappearance of TTV DNA did not affect the clinical course of chronic hepatitis favors the null hypothesis of no significant association of TTV with liver disease.

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