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Relation between viremia level and liver disease in patients with chronic HCV infection

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Abstract

AIM: To explore the relation between level of hepatitis C virus (HCV) viremia and HCV-related chronic liver disease.

METHODS: Serum HCV RNA was measured by competitive reverse transcription polymerase chain reaction (CRT-PCR) in 27 patients with chronic HCV infection.

RESULTS: Levels of serum HCV RNA were low (10^2-10^6 copies/50 μL serum) in patients with chronic HCV infection. Patients with chronic active hepatitis ($10^{5.739 \pm 0.25}$ copies/50 μL serum) and with cirrhosis ($10^{5.803 \pm 0.76}$ copies/50 μL serum) had higher levels of serum HCV RNA than patients with chronic persistent hepatitis ($10^{5.068 \pm 1.04}$ copies/50 μL serum) ($P < 0.05$). There was a positive relation between levels of serum HCV RNA and alanine aminotransferase.

CONCLUSION: All of these results suggest that viremia level is low in chronic HCV infection. HCV itself plays an important role in progress of chronic liver disease, and HCV replication is related to liver damage.

Key words: Hepatitis C virus; RNA, viral; Polymerase chain reaction; Hepatitis C

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INTRODUCTION

The pathogenesis of hepatitis C virus (HCV) infection is complex. Replication of HCV as well as host immune responses are involved in the liver cell damage that manifests in HCV infected individuals. Some studies have demonstrated that during the late stage of chronic liver disease, HCV still replicates actively[1]. We developed a competitive reverse transcription-polymerase chain reaction (CRT-PCR) for quantitative analysis of HCV RNA and investigated 27 patients with chronic HCV infection to explore the relation between HCV viremia level and chronic liver disease.

MATERIALS AND METHODS

Subjects

Twenty-seven patients (17 males and 10 females, aged 28-60 years) were hospitalized in our Department of Infectious Disease during the period from June 1993 to July 1995. All of these patients tested positive for anti-HCV and serum HCV RNA, and had shown abnormal levels of alanine aminotransferase (ALT) for at least 6 months. Thirteen of the cases were diagnosed as chronic persistent hepatitis (CPH), 10 as chronic active hepatitis (CAH) and 4 as cirrhosis, according to the criteria made at the National Conference on Viral Hepatitis, Shanghai, 1990. All 27 patients tested negative for hepatitis B surface antigen (HBsAg) and antibodies to the hepatitis A, D an E viruses (anti-HAV-IgM, anti-HDV IgM and anti-HEV IgM respectively). Serum samples were taken upon the first finding of HCV RNA positivity and stored at -20 ℃ until use.

Methods

RNA was extracted from 50 μL of each serum sample by using the acid quanidium thiocyanate phenol-chloroform method. HCV cDNA was synthesized from each extract by reverse transcription using primer 2 (Table 1). A competitive DNA plate was constructed by recombinant PCR. The restriction enzyme target site recognized by *BamH* I (GGATCC) was inserted into the HCV cDNA products by using primers 1 and 5, and primers 2 and 6 (Figure 1)[2,3]. Quantitative detection of serum HCV RNA was then carried out. The HCV cDNA from each serum sample was added to the competitive plate DNA ($10^{3\text{-}5}$ copies), respectively, and amplified by nested PCR using primers 1 and 2, and primers 3 and 4. The DNA product was then cut with endonuclease *BamH* I, electrophoresed through a 1.5% agarose gel, and stained with ethidium bromide; the staining pattern included three bands of 286 bp, 208 bp and 78 bp. The DNA was isolated from the 286-bp and 208-bp sized bands, respectively, by electro-osmosis and the OD_{260} value was detected. The number of HCV RNA copies in the 50 μL serum sample was calculated according to the formula: C1 = OD1/OD2 × C2 × 1.2, where C1 was the number of HCV RNA copies in 50 μL serum sample, C2 was the number of competitive plate copies, OD1 was the OD_{260} value of

Table 1 Sequences of primers located in the 5'-NC region of the hepatitis C virus genome

Primer NO.	Nuclear acid position	Polarity	Sequence (5'-3')
1	1-20	+	CGAGGCGACACTCCACCATAGAT
2	323-303	-	GAGGTGCACGGTCTACGAGACCT
3	10-32	+	CCACCATAGACTTCTCCCCTGT
4	296-270	-	CACTCTCGAGCACCCTCTCAGGCAGT
5	78*-60	-	CATGGATCCACTAACGCCATGGCTAGA
6	78*-96	+	ATGGGATCCATGATGGTCGTGCAGCCT

*The 10th base is the 78th nucleic acid in the sequence of the HCV genome. "GGATCC" is the site recognized by *BamH* I. HCV: Hepatitis C virus.

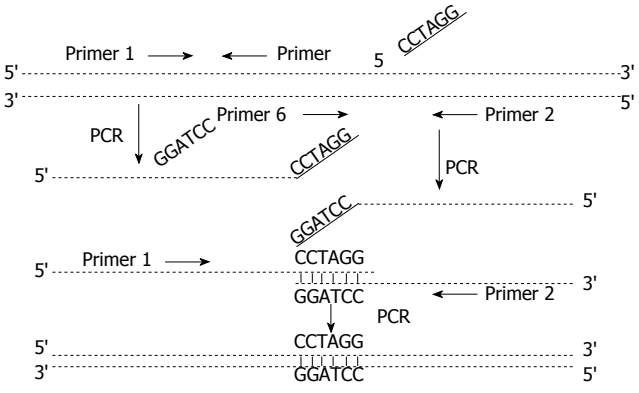

Figure 1 Competitive DNA plate construction by recombinant polymerase chain reaction.

Table 2 Comparison of serum hepatitis C virus RNA levels

Clinical classification	Number of cases	HCV RNA, copies/50 μL serum
CPH	13	$10^{5.068 \pm 1.04}$
CAH	10	$10^{5.739 \pm 0.25a}$
Cirrhosis	4	$10^{5.803 \pm 0.76}$

[a]$P < 0.05$ *vs* cirrhosis. HCV: Hepatitis C virus; CPH: Chronic persistent hepatitis; CAH: Chronic active hepatitis.

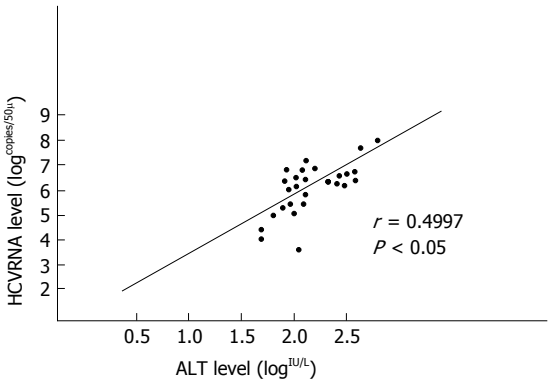

Figure 2 Relation between hepatitis C virus RNA and alanine aminotransferase.

DNA from the 286 bp band, OD2 was the OD_{260} value of DNA from the 208 bp band, and 1.2 was the transformation coefficient.

Anti-HCV was detected by second generation radioimmunoassay (RIA-2). HBsAg and IgM anti-HAV were detected by standard RIA. Anti-HDV and anti-HEV were detected by enzyme-linked immunosorbent assay.

RESULTS

HCV RNA level in serum

The serum levels of HCV RNA in patients with chronic HCV infection were low (10^2-10^6 copies/50 μL serum).

Serum level of HCV RNA in patients with different clinical classifications

The HCV RNA levels in patients with CAH and cirrhosis were much higher than those in patients with CPH ($P < 0.05$; Table 2).

Relation of HCV RNA level to ALT level

There was a positive correlation between levels of serum HCV RNA and ALT ($r = 0.4997$, $P < 0.05$; Figure 2).

DISCUSSION

In chronic HCV infection the viremia level is low. Bradley[4] conducted a chimpanzee infection experiment and found that serum titer of HCV was 10^2-10^4 CID/mL. A CID/mL of $10^{6.5}$ equates to 4 × 10^8 copies of HCV RNA/mL in serum[4]. Ulrich *et al*[1] used end-point dilution PCR to show that serum HCV RNA levels in patients with chronic hepatitis C were $10^{2\text{-}5}$ × 10^7 copies/mL. Hagiwara *et al*[5] used CRT-PCR to demonstrate that the serum HCV RNA was 10^4-10^9copies/mL. We obtained similar results in this study, detecting 2 × 10^3-2 × 10^7 copies/mL by the CRT-PCR technique. However, Jenison *et al*[6] demonstrated that the serum HBV DNA level in patients with chronic hepatitis B was 5 × 10^8-3 × 10^{10} copies/mL by dot blot hybridization, which is much less sensitive than PCR. It can be postulated that the viremia level in chronic HCV infection is significantly lower than that in HBV infection.

Replication of HCV is related to the progress of chronic liver disease. Our results show that serum HCV RNA levels in patients with CAH and cirrhosis are much higher than that in patients with CPH ($P < 0.01$). A similar result was reported by Kato *et al*[7], from a study in which they used CRT-PCR to detect HCV RNA in 36 patients with chronic liver diseases; specifically, the serum levels in patients with CAH ($10^{5.6 \pm 1.6}$ copies/50 μL) and with cirrhosis ($10^{6.0 \pm 1.6}$ copies/50 μL) were higher than that in patients with CPH ($10^{3.3 \pm 1.9}$copies/50 μL) ($P < 0.01$)[7]. Gordon *et al*[8] demonstrated that viremia level is positively related to liver histological score in patients with chronic HCV infection. Tsutsumi *et al*[9] studied the expression of NS5 antigen related to RNA-dependent RNA polymerase in liver, and found that the detection rate of NS5 antigen in patients with cirrhosis was higher than that in patients with CAH and CPH. Collectively, these data suggest that HCV itself plays an important role in the progress of HCV-related chronic liver disease.

HCV is known to replicate actively during the course of chronic HCV infection, and to replicate at high level even in the late stage of the disease. Moreover, HCV replication is well recognized as closely related to liver damage. It was demonstrated in this study that there is a positive correlation between the levels of serum HCV RNA and ALT in patients with chronic HCV infection ($r = 0.4997$, $P < 0.05$). Kurasaki *et al*[10] detected serum HCV RNA in 41 patients with chronic HCV infection by using PCR. Subgroup analysis of the 41 patients, by high-level HCV RNA (showing positive test results on the first amplification) and low-level HCV RNA (showing positive test results on the second amplification), indicated that the ALT in the high-level group was much higher than that in the low-level group (191.7 ± 103.4 μkat/L *vs* 98.4 ± 66.7 μkat/L, $P < 0.05$); additionally, all the patients showing ALT > 166.7 μkat/L belonged to the high-level HCV RNA group[10], suggesting that HCV may have a cytopathologenic effect.

In conclusion, the results of the current study demonstrate that the viremia level is low in chronic HCV infection, indicating that HCV replication is related to the progress of chronic liver disease and supporting the notion that HCV may have a cytopathologenic effect.

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