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Hepatitis C virus RNA genome in plasma of patients with non-A, non-B hepatitis

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Summary: Recently, the assay system of anti-hepatitis C virus antibody (HCV-Ab) was developed. However, there is no clinically useful method to detect hepatitis C virus (HCV) itself. The authors recently developed a method to detect the HCV-RNA genome in plasma using polymerase chain reaction (PCR). In the present study, the specificity of this assay in detecting HCV infection was investigated. Freshly obtained 1 ml plasma specimens from 100 patients with various liver diseases and from 11 control subjects were studied. In patients with non-A, non-B (NANB) hepatitis-related liver diseases, HCV-RNA was detected in 2 out of 7 cases of acute hepatitis, in 29 out of 31 cases of chronic hepatitis, in 17 out of 21 cases of cirrhosis and in 2 out of 6 cases of hepatocellular carcinoma. On the other hand, no HCV-RNA was detected in 15 cases of various types of alcoholic liver diseases, in 12 cases of hepatitis B related liver diseases, and in 11 controls. HCV-RNA was detected in 2 of 6 drinkers with chronic hepatitis. The prevalence of HCV-RNA was not closely related to a history of blood transfusions. These results suggest that our method for HCV-RNA is specific for HCV infection and HCV infection is the likely etiology of most chronic NANB hepatitis cases. The clinical usefulness of our method is illustrated by the fact that we were able to study 100 patients and needed only 1 ml plasma per HCV-RNA assay. *Gastroenterol Jpn 1991;26:42–46*

Key words: *HCV-RNA; hepatitis C virus; non-A, non-B hepatitis; polymerase chain reaction*

Introduction

Recently, the genome of the putative hepatitis C virus (HCV) was cloned and the assay system of anti-HCV antibody (HCV-Ab) was developed [1-3]. Using this system, HCV-Ab was detected in a large proportion of patients with chronic non-A, non-B (NANB) liver disease[2,4-8]. However, the presence of antibodies in blood does not necessarily indicate concomitant viremia or active virus infection of the liver. Detection of viremia is required for the accurate diagnosis of active HCV infection. However, there is no currently available method to detect HCV directly in small amounts of clinical material. The detection of HCV-Ab indicates past exposure to HCV but does not necessarily indicate active infection. To select appropriate patients for antiviral therapy the demonstration of HCV is necessary.

Recently, we developed a method to detect the HCV-RNA genome (HCV-RNA-NS5) in plasma using polymerase chain reaction (PCR). Our preliminary results indicated that HCV-RNA is found only in the plasma of those patients who have NANB hepatitis-related liver diseases[9]. In the present study, HCV-RNA was detected in the plasma of a larger number of patients, in a study carried out in order to clarify the specificity of HCV-RNA in HCV infection.

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Materials and Methods

Fresh plasma in order to avoid degeneration of virus RNA, were obtained from 100 patients with various liver diseases as well as from 11 healthy volunteers or patients without hepato-billary disease as controls. Of these 65 cases were NANB hepatitis; 7 had acute hepatitis (AH), 31 had chronic hepatitis (CH), 21 had liver cirrhosis (LC) and 6 had hepatocellular carcinoma (HCC). Diagnosis of these patients was performed histologically, except for 3 CH cases which were protracted cases of AH and HCC cases. HCC was diagnosed from clinical examinations, such as CT-scan, angiography, etc. Hepatitis B virus surface antigen (HBs-Ag), high titer anti-hepatitis B virus core antibody (anti-HBc Ab) and IgM anti-hepatitis A virus antibody (Anti-HA Ab) were all negative. HBs-Ag was positive in 14 cases; of these 8 had CH, 4 had LC and 2 had HCC. Fifteen patients had alcoholic liver disease (ALD); one of these had HCC, 7 of these had LC and 7 had alcoholic fibrosis. In these ALD patients, the hepatitis virus -A and -B markers were all negative. Six patients with chronic active hepatitis were heavy drinkers in whom hepatitis virus markers were all negative. In the control subjects, all liver tests were normal and had no history of blood transfusion.

HCV-RNA (NS5) in plasma was detected by the PCR method[9]. RNA was extracted by acid guanidine phenol method[10] from 1 ml of plasma and cDNA was synthesized by reversed transcriptase using a oligonucleotide primer synthesized based on the sequence of NS5 domain (position 6556-6956) of the prototype HCV as reported by Houghton et al[11]. The cDNA product was amplified by PCR with sense and antisense primers which were synthesized oligonucleotides complementary to the both ends of 401 base pairs (bp) DNA fragment of the prototype HCV genome. After amplification, one-tenth of the PCR products were subjected to electrophoresis through 4% polyacrylamide gel. The gel was stained with ethidium bromide to detect the amplification of 401 bp DNA fragment. This was the evidence for the presence of HCV genome (HCV-RNA-NS5) in plasma. To confirm the amplification of HCV genome, some PCR products were slot-blot hybridized with HCV cDNA in which nucleotide sequences were already determined[12].

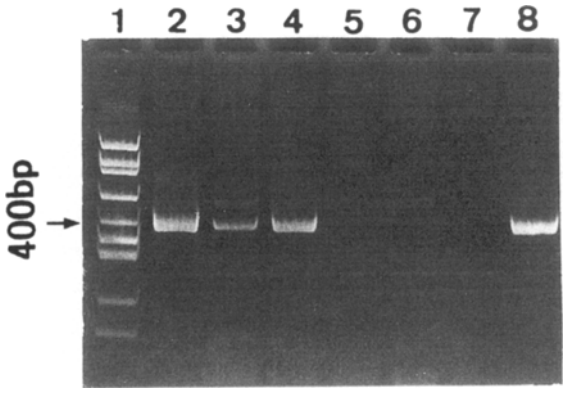

Fig. 1 Polyacrylamide (4%) gel electrophoresis of the PCR products.

The 401 base pair DNA fragments were found only in NANB hepatitis.

Lane 1: molecular weight marker, Lane 2, 4, and 8: chronic NANB hepatitis, Lane 3: acute NANB hepatitis, Lane 5: HBV liver cirrhosis, Lane 6: HBV chronic hepatitis, Lane 7: control.

Results

From the plasma of NANB-hepatitis patients, 401 bp DNA fragment were amplified by the PCR as shown in **Figure 1**. These products were hybridized with one of two different HCV cDNA in which nucleotide sequences were already determined[12], indicating that the PCR products of HCV cDNA did originate from the HCV-RNA genome in the patient's plasma (**Fig. 2**).

The incidence of HCV-RNA in the plasma of patients with various NANB liver diseases are shown in **Talbe 1**. In AH, plasma was obtained in the acute phase. In 2 of 7 AH patients (28.6%), HCV-RNA was positive. The two positive patients were acute post-transfusion NANB hepatitis. While the five negative patients were sporadic acute NANB hepatitis, only one of these 5 patients had a history of blood transfusion. The HCV-RNA was detected in 29 of 31 patients of CH (93.5%), in 17 of 21 patients of LC (81.0%) and in 2 of 6 HCC patients (33.3%). Only 10 of 29 (34.5%) HCV-RNA positive NANB CH patients

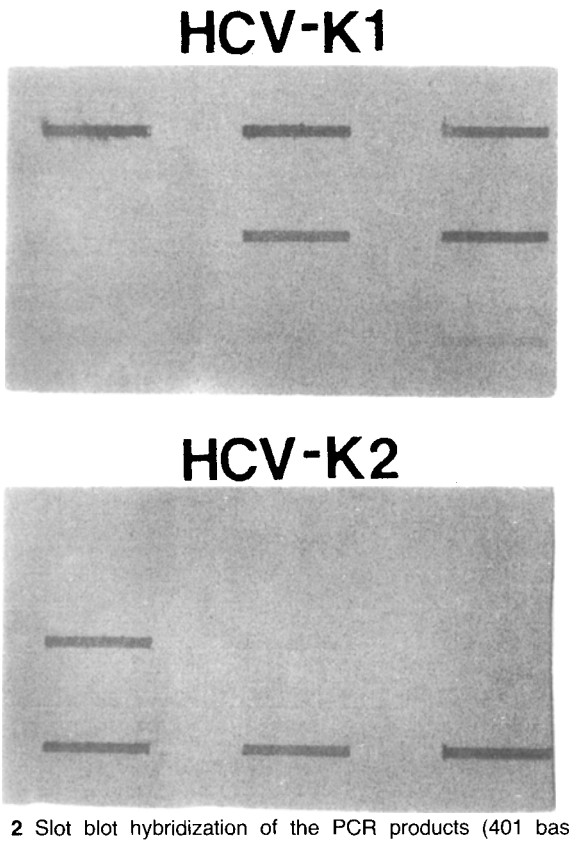

Fig. 2 Slot blot hybridization of the PCR products (401 base pairs DNA fragments) from chronic NANB hepatitis.
All PCR products hybridized to either of the two different cDNA probes (HCV-K1 and HCV-K2).

Table 1 Incidences of HCV-RNA in NANB-related liver diseases

Diseases	Cases	HCV-RNA	History of blood transfusion
Acute hepatitis	7	(+) 2 (28.6%)	2/2 (100%)
		(−) 5 (71.4%)	1/5 (20%)
Chronic hepatitis	31	(+) 29 (93.5%)	10/29 (34.5%)
		(−) 2 (6.5%)	2/2 (100%)
Liver cirrhosis	21	(+) 17 (81.0%)	7/17 (41.2%)
		(−) 4 (19.0%)	0/4 (0%)
Hepatocellular carcinoma	6	(+) 2 (33.3%)	1/2 (50%)
		(−) 4 (66.7%)	1/4 (25%)

Table 2 Incidences of HCV-RNA in other liver diseases

Diseases	Cases	HCV-RNA	History of blood transfusion
HBV liver disease	12	(+) 0 (0%)	
		(−) 12 (100%)	0/12 (0%)
Alcoholic liver disease	15	(+) 0 (0%)	
		(−) 15 (100%)	2/15 (13.3%)
Chronic hepatitis in drinkers	6	*(+) 2 (33.3%)	0/2 (0%)
		(−) 4 (66.7%)	1/4 (25%)
Controls	11	(+) 0 (0%)	
		(−) 11 (100%)	0/11 (0%)

*: $P<0.01$ compared with chronic NANB hepatitis by the X^2 test.

had a history of blood transfusion. The 2 HCV-RNA-negative NANB CH patients are protracted cases of acute post-transfusion NANB-hepatitis. Although abnormal values of liver tests continued for 2 years after blood transfusion, histological proof of CH has not yet been obtained. Seven of 17 (41.2%) HCV-RNA positive LC patients had a history of blood transfusion, but none of 4 HCV-RNA-negative patients had received blood transfusions. Each one of the HCV-RNA positive and negative HCC patient had a history of blood transfusion.

In none of the 12 patients with HBV-related liver disease was HCV-RNA detected and none of these patients had a history of blood transfusion. In all 15 patients with ALD, HCV-RNA was negative. Only two of these 15 (13.3%) had a history of blood transfusion. HCV-RNA was positive in 2 of 6 heavy drinkers (33.3%) with CH. One of the four HCV-RNA negative patients had a history of blood transfusion. HCV-RNA was not detected in any of the 11 control subjects (**Table 2**).

Discussion

In the present study, HCV-RNA was detected only in patients with NANB liver diseases. The incidence of HCV-RNA in chronic NANB hepatitis (CH) was very high. These results are compatible with the suggestion that HCV-RNA is highly specific for HCV infection and that HCV infection is the cause of most, if not all, chronic NANB hepatitis.

In AH, except for the fulminant type, HCV-Ab can be detected only during the convalescent stage, but not during the acute stage[6-8]. Therefore, diagnosis of acute HCV hepatitis based on

HCV-Ab is delayed for months after the onset of illness. On the other hand, HCV-RNA was detected during the acute stage of AH, and is the only method for the diagnosis of acute HCV hepatitis in the early acute stage. The authors detected HCV-RNA only in post-transfusion acute NANB hepatitis, but not in sporadic acute NANB hepatitis. This suggests a different etiology for post-transfusion and sporadic acute NANB hepatitis. However, a close relationship between a history of blood transfusion and HCV-RNA in chronic NANB liver diseases was not formed, indicating that blood transfusion is not the only factor in HCV infection. The incidence of HCV-RNA in NANB CH in heavy drinkers was clearly lower compared with that of NANB CH of non-drinkers. The number of patients is too small to draw any conclusions, but the incidence is compatible with the suggestion that either heavy drinking or another agent (non-B, non-C) is responsible for the development of CH in heavy drinkers.

The reported incidence of HCV-Ab (anti-C100-3) in chronic NANB liver diseases varies[2,4-8]. In most studies, the incidence ranged between 60-80% in CH, 50-75% in LC and 65-80% in HCC[2,4,5,7,8]. In the present study, the incidence of HCV-RNA both in CH and LC was higher than that based on HCV-Ab in these reports. On the other hand, incidence of HCV-RNA in HCC was lower than that reported with HCV-Ab.

Recently, Weiner et al[13] detected HCV-RNA from 10 liver and 1 plasma samples of human NANB liver disease and found some discrepancy in the results between HCV-RNA and HCV-Ab (anti-C100-3). They suggested that the prevalence of HCV infection may be underestimated if it is based on the detection of antibodies to C-100-3 alone. HCV-RNA in plasma is direct evidence of viremia. On the other hand, the relation between anti-C100-3 and viremia is unclear. HCV-RNA in plasma of some patients with NANB CH rapidly disappeared after treatment with interferon, indicating rapid decrease of HCV concentration in plasma. However, HCV-Ab remained positive in these treated patients (data not shown). Similar observations were made with HBV-DNA plasma concentrations after interferon treatment of chronic hepatitis-B. The low incidence of HCV-RNA in HCC may also be related to the concentration of HCV in blood. Therefore, discrepancies between HCV-RNA and HCV-Ab can be anticipated, depending on the presence of viremia. Different information is obtained by the determination of HCV-RNA and HCV-Ab for the diagnosis and treatment of HCV infection. Differences between subtypes of HCV genome in Japan and America[12] may be another problem for discrepancies concerning these markers, because the anti-C100-3 antibody test kit was produced by an HCV genome obtained from chimpanzee which was infected by HCV from an American patient. These results indicate the necessity for further studies to compare the results between HCV-RNA and HCV-Ab in the same patient in order to clarify the specificity of our assay system, and the relationship between NANB hepatitis and these virus markers.

Several reports described the detection of HCV genome in clinical material[13,14]. However, either large amounts of blood or liver tissue were used in these studies. In the present report, HCV-RNA was detected in 100 patients using only 1 ml of plasma, indicating that our method is simple enough for routine clinical use and sufficiently sensitive, because of very high prevalence of HCV-RNA in chronic NANB hepatitis.

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