Hepatocellular Carcinoma in a Patient with Liver Cirrhosis Associated with Negative Serum HCV Tests but Positive Liver Tissue HCV RNA

Tomoka ESAMI, Norihisa SUZUKI, Keiji YOKOYAMA, Kaoru IWATA, Makoto IRIE, Akira ANAN, Hidetoshi NAKANE, Makoto YOSHINANE, Shinya NISHIZAWA, Syuichi UEDA, Tetsuro SOHDA, Hiroshi WATANABE and Shotaro SAKISAKA

CASE REPORT

Abstract

A 70-year-old woman was admitted to our hospital because of a liver tumor. Laboratory data revealed mild liver dysfunction. Neither serum anti-HCV antibody nor HCV-RNA was detected. Computed tomography revealed a tumor lesion measuring 2 cm in diameter within the liver. Histological examination of the tumor revealed moderately differentiated hepatocellular carcinoma while the non-tumorous liver tissue demonstrated liver cirrhosis. By the RT-PCR method, HCV-RNA was detected from the non-tumorous liver tissue. We herein report a very rare case of hepatocellular carcinoma in a patient with liver cirrhosis associated with negative serum HCV findings, but positive finding for liver tissue HCV RNA.

Key words: hepatocellular carcinoma, HCV-RNA

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies throughout the world. In Japan in 2000, HCC was the 3rd leading cause of death from malignancy in men, and the 6th in women (1). In Japan, about 70% of HCC cases are associated with hepatitis C, and accordingly hepatitis C is extremely important as a contributory liver disease leading to the development of HCC (2). For a diagnosis of hepatitis C, anti-HCV antibody and serum HCV-RNA are both quite reliable, although false-negative cases are occasionally encountered (3, 4). We herein report a case of HCC in a patient who tested negative for serum HCV, but positive for HCV-RNA in the liver tissue.

Case Report

In April 2002, a 70-year-old woman who had been previously diagnosed as exhibiting liver dysfunction at 60 years of age, was admitted to the Department of Dermatology in our hospital because of genital skin cancer. Since liver dysfunction was evident, she underwent abdominal computed tomography examination and a mass lesion measuring 2 cm in diameter was found in the right lobe of the liver. On May 24, after undergoing surgery for the skin cancer, she was admitted to our department for the treatment of a liver tumor. The patient had never received a blood transfusion and had no habit of drinking alcohol. On admission, physical examination demonstrated edema of the bilateral legs and a surgical scar on the genital skin.

Laboratory data (Table 1) revealed elevated serum levels of aspartate aminotransferase and lactate dehydrogenase. Hepatitis B surface antigen was negative. Neither serum anti-HCV antibody nor HCV-RNA were detected. Anti-nucleus antibody was positive, but the international autoimmune hepatitis score (5) was 7 points. Antimitochondrial antibody was negative. Ultrasonography and computed tomography (Fig. 1) revealed an enhanced tumor measuring 2 cm in diameter within the right lobe of the liver. Needle liver biopsy was performed. Histological examination of the liver tumor revealed atypical cells arranged in a trabecular pattern (Fig. 2A), while the non-tumorous liver tissue revealed regenerative pseudolobules surrounded by fibrous septa with inflammatory cell infiltration (Fig. 2B). Pathological diagnosis was moderately differentiated HCC with liver cirrhosis. Transcatheter arterial embolization and percutaneous radio-frequency ablation therapy were performed for the liver...
In an effort to ascertain the cause of the liver cirrhosis, liver tissue was tested for the viral hepatitis gene. For PCR for HBV-DNA, using primers (5’-biotin-GTT CAI GCC TCC AAG CTG TG and 5’-TCA GAA GGC AAA AAI GAG AGT AACT), the pre-core/core regions (base: 1864–1967) of HBV-DNA were amplified. And HBV-DNA was not detected by this method.

Although, by the RT-PCR method, HCV-RNA was detected at 1.2×10^2 copies per 10 mg of non-tumorous liver tissue.

The HCV-RNA test of the liver tissue from our patient was performed in the laboratory of SRL Inc. (Tokyo, Japan). The method used was the quantitative PCR method using the real-time monitoring technique (6, 7). RNA was extracted from 100μl of serum or 10 mg of liver tissue by the acid guanidinium thiocyanate-phenol-chloroform method (8).

Reverse transcription for the synthesis of cDNA and PCR was performed using 5 μl of RNA extracted from either serum or liver tissue. Twenty μl of the reaction mixture containing 25 mM Mn (OAc)₂, 20 mM dUTP, 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 11.0 pmol of each primer, 11.0 pmol of Tag Man Probe and 2.5 U/μl rTth DNA polymerase were incubated at 60°C for 30 minutes. PCR was carried out

**Table 1. Laboratory Data on Admission**

<table>
<thead>
<tr>
<th>Peripheral blood</th>
<th>Fe</th>
<th>134 μg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>306×10⁴/μl</td>
<td>UIBC</td>
</tr>
<tr>
<td>Hb</td>
<td>11.0 g/dl</td>
<td>Ferritin</td>
</tr>
<tr>
<td>Hct</td>
<td>31.5%</td>
<td>Coagulation</td>
</tr>
<tr>
<td>WBC</td>
<td>2,800/μl</td>
<td>PT</td>
</tr>
<tr>
<td>Plt</td>
<td>7.5×10⁴/μl</td>
<td>Immunology</td>
</tr>
</tbody>
</table>

Serum chemistry

| T. P. | 6.7 g/dl | IgG | 1,790 mg/dl |
| T. Bil | 1.8 mg/dl | IgA | 624 mg/dl |
| γ-glob | 1.9 g/dl | IgM | 186 mg/dl |
| AST | 59 IU/l | ANA | 40 dil |
| ALT | 25 IU/l | AMA | (–) |
| LDH | 685 IU/l | RF | <20 dil |

Alcohol markers

| ALP | 327 IU/l | Virus markers |
| ALT | 110 IU/l | HBs-Ag | (–) |
| T. Chol | 186 mg/dl | HBc-Ab | (–) |
| TG | 62 mg/dl | HCV-Ab | (–) |
| FBS | 76 mg/dl |


Figure 1. Computed tomography reveals an enhanced tumor measuring 2 cm in diameter in the right lobe of the liver.

Figure 2. A) The liver tumor shows atypical cells arranged in a trabecular pattern. B) Non-tumorous liver tissue shows regenerative pseudolobules surrounded by fibrous septa with inflammatory cell infiltration (HE A; ×200, B; ×100).
for 53 cycles following reverse transcription with denaturation for 20 seconds at 95°C and annealing and extension for 1 minute at 62°C. The primer was selected from the 5’ non-structural region of the HCV genome, the sense primer was 5’CGGGAGGCCATAGTG3’, and the anti-sense primer was 5’AGTACCACAGGCTTTCG3’. By use of the fluorescence-labeled Taq Man Probe, quantitative estimation was performed.

**Discussion**

The current patient suffered from HCC associated with liver cirrhosis. As for the cause of the liver cirrhosis, alcohol was not involved since the patient had no habit of drinking alcohol. According to serological tests and liver histological findings, it was clear that autoimmune hepatitis, primary biliary cirrhosis, nonalcoholic steatohepatitis (NASH) and hemochromatosis were also not the probable causes. Although Wilson’s disease and type I autoimmune chronic active hepatitis were not ruled out, the background liver disease may be said as “cryptogenic liver cirrhosis”.

Hepatitis B was not a probable cause since the results of hepatitis B surface antigen, hepatitis B core antibody and liver tissue HBV-DNA were all negative. On admission, it was also thought that hepatitis C was not a probable cause because the results of third generation anti-HCV antibody and serum HCV-RNA were both negative. However, HCV-RNA was detected in the liver tissue. In this study, the primer was designed for the HCV-RNA (+) chain, and HCV-RNA (-) chain, and so the mediator for reproduction of HCV-RNA was not evaluated. Therefore, the growth of HCV in the liver tissue was not demonstrated. And this was the first indication that the patient was infected with hepatitis C. She had never received any antiviral therapy such as interferon. Although she had been diagnosed with liver dysfunction ten years earlier, the findings of the liver function test and viral hepatitis test at that time are not known.

Anti-HCV antibody is a reliable test for the diagnosis of hepatitis C. However, false-negative results can occasionally be obtained. Saldanha and Minor (3) studied 95 plasma samples with negative anti-HCV antibody and reported that HCV-RNA was detected in 5 samples (5%). False-negative results of anti-HCV antibody tests can appear during the window period of HCV infection and can also appear due to human and/or operational error. Moreover in children, anti-HCV antibody-negative chronic hepatitis C virus infection has been reported. Januszkiewicz-Lewandowska et al (4) followed 33 children with cryptogenic hepatitis for a mean of 2.5 years and reported that during this period, 17 children showed positive findings for serum HCV-RNA, but only 8 of the 17 children showed positive findings for anti-HCV antibody. In two other children, liver tissue was positive for HCV-RNA, but negative for serum HCV markers.

In cases where there are negative findings for anti-HCV antibody, the HCV-RNA test is thought to be reliable. However, false-negative results for serum HCV-RNA have been reported in cases of cryoglobulinemia (9). There have also been studies demonstrating that PCR tests for HCV-RNA show differing sensitivity depending on the samples measured. Schmidt et al (10) studied whole blood and plasma samples from 15 patients with cryptogenic hepatitis and reported that HCV-RNA was detected in 10 of the whole blood samples but only in 5 of the plasma samples. Das et al (11) studied 10 patients with positive results for HCV-RNA in liver tissue and reported that serum HCV-RNA was detected in 7 cases, whereas anti-HCV antibody was detected in only 4 cases. According to these studies, the order of sensitivity of HCV tests would seem to be liver HCV-RNA, whole blood HCV-RNA, plasma HCV-RNA, followed by anti-HCV antibody. Therefore, screening of only anti-HCV antibody and serum HCV-RNA is not sufficient for a definite diagnosis of hepatitis C.

On the other hand, in very rare cases, serum HCV markers have been demonstrated to disappear during the natural course. Feucht et al (12) followed up 4,110 patients with positive anti-HCV antibody findings for 2 years. During this period, only one case (0.04%) spontaneously lost serum HCV-RNA positivity and subsequently anti-HCV antibody positivity.

In the current case, three months after being discharged from our hospital, the patient was re-tested for anti-HCV antibody and serum HCV-RNA and the results of both were again negative. Therefore, the findings in this patient could not have been due to recent HCV infection or errors in testing. Since liver function tests and viral hepatitis tests were not performed in the past, the course of our patient is unclear. Our patient is considered to have suffered from HCV-related liver cirrhosis but lacks serological response.

With regard to the cause of liver cirrhosis in Japan in 1999, it was found to be hepatitis C in 65% of cases, hepatitis B in 12%, alcoholism in 13% and cryptogenic hepatitis in 4.3% (13). Recently, many studies have been performed for patients with cryptogenic hepatitis and the importance of occult hepatitis B and NASH has been emphasized. In Hong Kong, a high prevalence of occult hepatitis B among patients with cryptogenic hepatitis has been reported (14). In the United States and Europe, NASH-related diseases including obesity, diabetes mellitus and hyperlipidemia are common among patients with cryptogenic hepatitis. Moreover, the association of HCC with these cases has also been reported (15, 16).

The present case would have been regarded as cryptogenic hepatitis if the liver HCV-RNA test had not been performed. We therefore recommend that when considering the cause of cryptogenic cirrhosis, the possibility of occult hepatitis C should always be taken into consideration.

**Acknowledgements**: The English used in this manuscript was revised by Miss K. Miller (Royal English Language Centre, Fukuoka, Japan).
References