Long-term Follow-up of a Patient with Hepatocellular Carcinoma Associated with Triple Hepatitis Virus (HBV, HDV, HCV) Infection

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Abstract

A 69-year-old Japanese man with hepatocellular carcinoma (HCC) associated with triple hepatitis viruses [hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV)] infection is reported. The patient had a past history of intravenous drug abuse and a tattoo on his back. A liver biopsy, performed in November 1989, showed HCC associated with cirrhosis. HBsAg and anti-HD antibody had been detected repeatedly starting in August 1984 and anti-HCV antibody was detected in 1990. By indirect immunoperoxidase staining the HD antigen was detected in the nuclei of hepatocytes of biopsy specimens and noncancerous liver cells obtained from autopsy specimens.

Liver cirrhosis associated with triple hepatitis virus infection developed to hepatocellular carcinoma, and transcatheter arterial embolization treatment for HCC was effective. Despite having HCC and cirrhosis, the patient lived well beyond the expected time.

Introduction

The hepatitis D virus (HDV) is a defective, hepatotropic RNA virus which is only capable of synthesizing infectious particles in the presence of hepatitis B virus (HBV)1,2). HDV can occur as a co-infection with HBV, which usually causes acute hepatitis, or as a superinfection in an HBV carrier3).

The frequency of hepatitis delta virus infection in Japan is 1% to 2% among HBV carriers, which is lower than that reported in the United States and Europe4,5). It has been reported that in the US and Europe, HDV infection is clinically similar to the ordinary types of acute or chronic hepatitis, but can lead to a more severe condition. In Japan, however, the relationship between HDV and liver diseases has not yet been established because patients with HDV are rare.

We describe the course of a patient with hepatocellular carcinoma (HCC) and evidence of HBV and hepatitis C virus (HCV) infection in whom serum anti-HD antibody was repeatedly detected for an extended period.

Case study

The patient was a 69-year-old Japanese man whose chief complaint was general fatigue. The
patient injected methamphetamine intravenously at a dose of 15 to 30 mg/day for about 18 months from 1949 to 1950 and also had a tattoo on his back. The patient’s wife had received a blood transfusion during surgery for an ectopic pregnancy at the age of 27 years and after a spontaneous abortion at the age of 34 years. She was negative for hepatitis B surface (HBs) antigen, but positive for HCV RNA. Her children were negative for both HBs antigen and anti-HCV antibody. In 1975 the patient was referred to the outpatient clinic of the Department of Internal Medicine, Kure National Hospital because of general fatigue and abdominal distension, and was diagnosed as having liver cirrhosis.

Between February 1985 and November 1994, the patient was admitted to the hospital several times. A liver biopsy performed in November 1989 showed HCC and cirrhosis. Sclerosing therapy was conducted three times for esophageal varices, and transcatheter arterial embolization (TAE) was carried out three times for HCC during this time period (Fig. 1).

In February 1995, the patient developed general fatigue and anorexia, and was admitted to the hospital. Physical examination revealed a liver palpable 4 cm below the right costal margin.

Laboratory findings on admission were increases in aspartate aminotransferase (AST) (127 IUL), γ-GTP (170 IU/L), and ZTT (41.6 KU). The γ-globulin level was increased (39.6%) and the Ch-E level was decreased (2189 IU/L). As for serum tumor markers of HCC, α-fetoprotein (AFP) (89.0 ng/ml) and PIVKA-II (1.59 AU/ml) levels were increased. The findings were consistent with the diagnosis of hepatocellular carcinoma associated with cirrhosis. Regarding hepatitis virus markers, the patient tested positive for HBsAg, anti-HD and anti-HCV but tested negative for HBV DNA. However, he tested positive for HCV RNA which was measured using an AMPLICOR HCV-Monitor (NIPPON Roche Co. Ltd., Tokyo).

In September 1995, computed tomography revealed recurrence of HCC in the right lobe of the liver, and markedly high serum levels of AFP were detected. In mid-September of the same year, subjective symptoms worsened. In January 1996 the patient died (Fig. 1). Autopsy of the entire body (excluding the head) performed approximately 1.5 hours after death revealed cirrhosis (active form, 900 g) and hepatocellular carcinoma (multiple, nodular, moderately-differentiated hepatocellular carcinoma).
Liver biopsy and autopsy specimens were fixed in periodate-lysine-paraformaldehyde (PLP) solution. A portion of the liver tissue was stored at −80°C until immunochemical examination. Using frozen sections of liver biopsy and autopsy specimens, localization of HBsAg, HDAg was determined by indirect immunoperoxidase staining. HBsAg was localized in the cytoplasm of hepatocytes in the specimen obtained at autopsy using anti-HBs antibody (Dako Japan, Co. Ltd., Tokyo) (Fig. 2a).

Since it is difficult to produce anti-HD antibody, a panel of sera known to have high titers (1: 10,000) of circulating anti-HD antibody as determined by solid-phase blocking radioimmunoassay was used as the first antibody6). PLP-fixed biopsy and autopsy specimens were processed according to an indirect immunoperoxidase method to detect HD antigen using anti-HD antibody after treating the section with human anti-HBc antibody produced in rabbits (Dako Japan, Co. Ltd., Tokyo). A preliminary blocking of endogenous peroxidase was carried out using the method of Zehr7). The deplasticized sections were overlaid with anti-HD antibody serum at a 1: 1024 dilution and incubated in a moist chamber at room temperature for 1 hour. Thereafter, the sections, were overlaid with horseradish peroxidase-conjugated rabbit anti-human IgG (Tago Inc., Kyoto, Japan) at a 1: 32 dilution and incubated for 1 hour in a moist chamber at room temperature. HBc antigen was completely blocked by anti-HBc antibody, but the HD antigen was expressed in the nuclei of hepatocytes obtained from biopsy specimens and noncancerous liver cells obtained from autopsy specimens (Fig. 2b).

Discussion

HDV infection often causes severe disease, resulting in cirrhosis in many patients with acute hepatitis8). Previous studies in Europe and the United States have shown a high prevalence of delta markers among patients with fulminant HBsAg-positive hepatitis (21 to 50%). Such severe disease is likely to involve additive damage caused by the two viruses. Discrepancies among previous studies regarding severity of disease due to HDV may reflect geographic differences between strains9)-12).

Long-term follow-up of several Japanese patients with hepatitis delta virus has indicated a relatively favorable prognosis. Yasui et al. have reported observations of a patient positive for

Fig. 2 Localization of hepatitis B surface antigen and hepatitis D antigen in the noncancerous liver cells.

a: HBsAg was seen in the cytoplasm of liver cells. b: HD Ag was detected in the nuclei of hepatocytes, never in the cytoplasm (indirect immunoperoxidase stain: original magnification ×400).
anti-HD over a period of 11 years; chronic hepatitis had caused cirrhosis and hepatocellular carcinoma. Analysis of a liver biopsy specimen obtained 7.5 years after onset demonstrated the δ antigen in hepatocytes, but the antigen could not be detected in subsequent autopsy specimens. In our patient, HD antigen was expressed in hepatocytes in biopsy specimens as well as in noncancerous liver cells in autopsy specimens. HBV infection is closely linked to development of chronic liver disease. Genomically integrated HBV DNA has been found in the majority of HCCs arising in chronic HBV carriers. Our patient’s serum was negative for HBV DNA. HCC occurring in the presence of HDV Ag appears to differ in a number of ways from HCC associated with HBsAg. In future studies, we would like to identify the HDV strain of the present patient to better understand the implications of the case.

The overall positivity rate for anti-HD antibody among chronic HBsAg carriers has been estimated at 5.2% (10/194), while in the same study none of the 11 patients with HCC were positive for HDV. The fact that HCC seems to occur less frequently in HBsAg carriers when they are superinfected with HDV may be attributable to inhibition of hepatitis B or, alternatively, to rapid progression of fatal liver disease before cancer can develop.

Transmission of hepatitis delta virus may be influenced by blood transfusions and social factors such as habitual drug injections. Our patient was an intravenous drug abuser who also had undergone tattooing on his back. Most likely, the patient was infected with HDV through needle sharing. Abnormal results of liver function tests had been evident since 1985, and subsequently the patient developed hepatocellular carcinoma. HDV superinfection and HCV infection in this HBV carrier may alternatively have been related to several types of exposure: the patient’s wife was positive for HCV RNA, and she may have been infected from the blood transfusions at ages of 27 and 34 years.

Superinfection with non-A, non-B viruses is known to interfere with HBV activity, as indicated by transient disappearance of markers of HBV replication and reduction in serum HBsAg concentration. Bradley et al. have reported that persistent non-A non-B hepatitis infections efficiently suppressed or inhibited replication of two different hepatotropic viruses in chimpanzees, while another group found that HDV enhanced HBV clearance in human carriers. The patient was negative for HBV DNA. Interference by HCV with HBV replication is evident from decreasing levels of HBV gene products in serum during HDV infection. HCV superinfection, therefore, appears to reduce replication of HBV.

In the present patient with cirrhosis associated with triple hepatitis virus infection (HBV, HDV and HCV), HCC developed but TAE treatment was effective. Despite having HCC and cirrhosis, the patient lived well beyond the expected time.

References
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長期経過観察した肝炎ウイルス（HBV, HDV, HCV）陽性肝細胞癌の1例

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要　旨

本邦でのデルタ肝炎の長期経過観察例は少なく、肝疾患との関係も判然としない。B型肝炎ウイルス（HBV）、デルタ肝炎ウイルス（HDV）およびC型肝炎ウイルス（HCV）抗体陽性肝細胞癌を約7.5年間経過観察した1例を経験した。

[症例] 69歳、男性、1949年から約1.5年間、塩酸メタンフェタミン15mg〜30mg/日、常時していた、刺激（+）。1975年より肝機能障害で某院に通院、1984年2月肝硬変のため、国立岡病院に紹介された。1989年11月腹腔内鏡、肝生検で肝硬変を伴う肝細胞癌と診断した。以後肝動脈塞栓術を3回行つた。1995年2月、全身倦怠、食欲不振のため入院。7月頃より肝腫瘤マーカー（AFP, PIVKA-II）の上昇、全身状態が徐々に悪化し1996年1月死亡した。HBs抗原、抗HDV抗体は1984年8月、抗HCV抗体は測定開始時の1990年より持続陽性であった。また、肝生検、剖検材料より非癌組織中にデルタ抗原を証明した。

本症例はHBV、HDV、HCVの三重感染に発症した肝硬変に肝細胞癌を併発したにもかかわらず、肝動脈塞栓術の効果により长期生存したと思われる。