Chronic Hepatitis Infected with Hepatitis GB Virus Type C/ Hepatitis G Virus Presenting as Non-Alcoholic Steatohepatitis

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A 68-year-old man with moderate liver dysfunction diagnosed with atypical pneumonia showed serum alanine aminotransferase and γ-glutamyltranspeptidase levels which revealed a sustained abnormality over six months. Hepatitis GB virus type C/hepatitis G virus was demonstrated in his serum by reverse transcription-polymerase chain reaction. Liver histology showed steatohepatitis typically observed in alcoholic hepatitis without a remarkable drinking history. This case suggests that hepatitis GB virus type C/hepatitis G virus may induce chronic hepatitis and that there may be cases with chronic hepatitis induced by this virus in patients who have been diagnosed with alcoholic liver disease, even in cases with typical histology of alcoholic hepatitis. (Internal Medicine 36: 283-288, 1997)

Key words: alcoholic hepatitis, atypical pneumonia, liver biopsy, reverse transcription-polymerase chain reaction

Introduction

A group of flavi-like viruses has been reported to be a potential cause of hepatitis in tamarin (1). The role of two of these viruses, GB virus (GBV) type A and GBV type B, in human viral hepatitis has not been determined; however, a third agent of non-A-E hepatitis in human hepatitis, namely hepatitis GB virus type C/hepatitis G virus (HGBV-C/HGV), has been reported (2). The putative helicase domain of HGBV-C was found to have amino acid sequence homology with the hepatitis C virus (HCV) (3) and viral RNA has been detected by reverse-transcription polymerase chain reaction (RT-PCR) in patients with fulminant hepatitis (4), drug abusers with chronic hepatitis C (5), and even in plasma pools (6).

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Thirty serum samples stored at −80°C derived from patients with non-A-C acute, and chronic hepatitis were examined to determine whether HGBV-C/HGV could be detected under the guidance of the non-A, non-B Hepatitis Study Group in the Ministry of Health and Welfare of Japan. One HGBV-C/HGV-positive sample was discovered. We describe here a case of HGBV-C/HGV-induced chronic non-A-E hepatitis.

Case Report

A 68-year-old man was admitted to our hospital in March 1994 because of fever, dry cough and general fatigue. He had undergone medical examinations including laboratory examinations once or twice a year until his admission. Multiple silent gallstones (1–3 mm in diameter) were detected from ten years previously by abdominal ultrasound without evidence of inflammation. He underwent an operation for right inguinal herniation when he was 5 years old, but had no previous history of liver dysfunction, blood transfusion, intravenous drug abuse, or tattoo, and he had not taken any medication before. His body weight was 69 kg and height was 165 cm. His X-ray examination showed pulmonary infiltration and pleural effusion in the left lower lobe (Fig. 1), and his serum alanine aminotransferase (ALT) level was 135 IU/l. Laboratory date on his admission are shown in Table 1. He was diagnosed with atypical pneumonia and was treated with erythromycin. His pneumonia subsequently improved, but serum ALT rose to about 250 IU/l and the γ-glutamyl transpeptidase (γ-GTP) level was 237 IU/l (normal range: ALT, 8–41 IU/l; γ-GTP, 11–95 IU/l at 37°C). Because the increase in ALT level was considered to be drug induced, erythromycin was changed to clarithromycin. The patient recovered completely from atypical pneumonia, and his serum ALT level decreased to 50 IU/l. He left the hospital in April 1994. He has been followed up in our outpatient clinic once a
month, and his serum ALT and γ-GTP levels have shown mild but persistent abnormalities. He underwent cholecystectomy without any complication in July 1994, because his serum enzymic abnormalities had continued. However, the abnormal levels of serum ALT and γ-GTP have continued. Diabetes, a history of exposure to drugs, hypothyroidism, tests for Epstein-Barr virus, cytomegalovirus, toxoplasma and autoimmune hepatitis were negative and metabolic diseases (Wilson’s disease, hemochromatosis, alpha-1-antitrypsin deficiency) were excluded by specific tests. In October 1995, HGBV-C/HGV was found to be positive in his serum taken in May 1995 by a detection kit using RT-PCR (GBVc-RNA detection kit Ns, Dainabot Co., Ltd., Tokyo) and also by RT-PCR according to a method described previously (4). Briefly, total RNA was extracted from 100 μl of his serum and cDNA was amplified by PCR after reverse transcription using the antisense primer “G9” (5’-TCYTTG ATG ATD GAACTG TC-3’; Y=T or C; D=A, G, or T). The primer pair used to amplify a putative helicase region of HGBV-C/HGV was “G9” and “G8” (5’-TAT GGG CAT GGHATH CCYCT-3’; H=A, C, or T; Y=C or T). After the first round 35-cycle PCR with “G9” and “G8”, a second-round 30-cycle PCR was performed with the primer “G8” and “G11” (5’-TCYTTA CCC CTRTAATAG GC-3’; Y=C or T; R=A or G). Each cycle included denaturation at 94°C for 30 second, anneal-
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...ing at 58°C for 30 second, and extension at 72°C for 45 second. The expected size for the semi-nested PCR products to be derived from HGBV-C/HGV genomic RNA was 140 bp (Fig. 2).

To confirm the detection of HGBV-C/HGV, we conducted another RT-PCR using primers derived from the 5'-UTR, which were highly conserved between HGBV-C and HGV, and distant from flaviviruses, pestiviruses and HCV (7). These primers were kindly donated by Dr. Masashi Mizokami (Second Department of Internal Medicine, Nagoya City University, Medical School). RT-PCR was performed according to the protocol proposed by Dr. Mizokami, and the expected PCR product was detected on 3% agarose gel (data not shown). Needle biopsy of the liver was performed in November 1995. The clinical course is shown in Fig. 3.

Histological examination showed periportal fibrosis, intralobular fibrosis with a pericellular extension, and ballooning with macrovesicular fatty change of moderate degree. Several hepatocytes containing Mallory bodies were observed and foci of hepatocellular degeneration with neutrophilic infiltration

Figure 2. Agarose gel electrophoresis of PCR products. Lane 1: molecular markers (100 bp ladder), Lane 2: negative control, distilled water was used in place of serum in the extraction of RNA, and cDNA synthesis and PCR were carried out, Lane 3: PCR product of the sample from a patient with nonB-nonC chronic hepatitis, Lane 4: PCR product of the sample from the patient of this case.

Figure 3. Clinical course of the patient. ALT: alanine aminotransferase, γ-GTP: gamma-glutamyltranspeptidase.

- Glutathione 300 mg, glycrrhizin
- Ursodesoxycholic acid 600mg

Liver biopsy

ALT (8-41 IU/l, normal)
γ-GTP (11-45 IU/l, normal)
were sometimes noticed (Figs. 4–6). Mild lymphocyte infiltration was observed in both portal and central areas. Inflammatory change was more severe in the centrolobular area (zone 3 of Rappaport). These histologic features resemble those of alcoholic hepatitis, but the injury seems somewhat less severe. He had not drunk more than one can of beer (350 ml) daily for the past 30 years until he was admitted to our hospital at which time he stopped drinking completely. He retired from business eight years before his admission and he has never gone outside for drinking after his retirement. His family including his son who is a medical doctor confirmed his history of drinking.

**Discussion**

It is well known that the liver is the organ most severely affected by excessive alcohol intake (8). Three obligatory features have been defined as essential for the histologic diagnosis of alcoholic hepatitis (9). The first is liver cell damage, typically following degeneration with areas of necrosis, the second is inflammatory cell infiltration, predominantly polymorphonuclear leukocytes, and the last is fibrosis, both with pericellular and perivenular. The association with Mallory’s bodies is another frequent characteristic of alcoholic hepatitis (9–11). All these features were observed in the present case. Our patient’s alcohol consumption, however, had been very modest and his liver biopsy was performed 8 months after the cessation of drinking, suggesting that histological changes in the liver were expected to be none or at least minimal, unlike the features of his biopsy specimen. Risk factors were ruled out from his laboratory data.

Although less common, histologic features obtained in the present case including Mallory’s bodies have been observed in non-alcoholic liver pathologic condition (12–28). This condition usually is referred to as non-alcoholic steatohepatitis (29). In the absence of alcohol, steatohepatitis has been reported with cirrhosis (12–16), nutritional abnormalities (17–22), metabolic dysfunction (23, 24), and drug toxicity (25–28). In one large series of 543 cases of histologic alcoholic hepatitis, 49 cases were not clinically related to alcoholism (30). Conte et al (31) described five cases of non-alcoholic steatohepatitis in the absence of known causes of liver damage. The findings of the present case were consistent with non-alcoholic steatohepatitis, in which Mallory’s bodies, relatively slight steatosis, and portal mononuclear infiltration are observed; a rare condition with potential progression to cirrhosis in a minority of cases. The drugs chiefly responsible for the entity are perhexiline (26, 32, 33) and aniodarone (25, 34–36), and Mallory’s bodies can be produced by diethylstilbestrol (28, 37), nifedipine (38) and ethanol. No such drugs were administered to this case and he received only erythromycin and clarithromycin, to our knowledge, which have not been reported to cause steatohepatitis.

Yoshiba et al (4) reported that three of six patients with non-A-E fulminant hepatitis had HGBV-C genomes in their serum and suggested that HGBV-C is important in the etiology of fulminant hepatitis. However, whether HGBV-C/HGV is one of the causative agents of non-A-E fulminant hepatitis has not
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been defined (39–41). Since HGBV-C/HGV is considered to be a member of Flaviviridae and shares the same ancestor with GB virus A, B and HCV (2, 3, 42), it has been postulated that HGBV-C/HGV may be a cause of chronic hepatitis. The detection of serum HGBV-C/HGV RNA by RT-PCR has been reported in a proportion of patients with chronic hepatitis C or B virus (HBV) infection and in patients with histories of intravenous drug use or blood transfusion (5, 43), however, such dual infection was not associated with severity of chronic liver diseases, compared to single HBV or HCV infection (44). Among Japanese patients with chronic liver diseases, 10/203 (4.7%) showed serum HGBV-C/HGV RNA by RT-PCR, but they were all coinfected with HBV or HCV (7). These conditions suggest that there is a low prevalence of HGBV-C/HGV infection in Japanese patients with chronic liver diseases, however, a high proportion of patients with HGBV-C/HGV infection have chronic HCV or HBV infection.

Different from these conditions, in an Italian study, a proportion of patients with non-A-E chronic hepatitis has been shown to be positive for serum HGBV-C/HGV (45). In the present study, serum HGBV-C/HGV RNA was detected by RT-PCR in a Japanese case with non-A-E chronic hepatitis. His liver biopsy specimen showed steatohepatitis which is consistently seen in patients with alcoholic hepatitis. However, the patient had no risk factors such as nutritional abnormalities, metabolic dysfunction, history of taking causative drugs, and much drinking. The one and only possible cause of his liver injury may be HGBV-C/HGV infection. Relatively severe hepatic dysfunction was noted at his admission to our hospital. It is difficult to explain this liver damage exactly, but if he had suffered from steatohepatitis before the admission, hypoxia (pO2 51–65 mmHg) which was caused by atypical pneumonia possibly exacerbated the hepatitis already present in his liver. Another possibility is that asymptomatic cholecystitis induced by gallstones exacerbated the liver cell damage.

After the discovery of HCV, many alcoholic patients have been shown to be infected with HCV (46). Uchimura et al (47) histologically compared chronic hepatitis C with alcoholic liver disease, and histological similarities were suggested between these two types of diseases. However, steatohepatitis is a rare condition, which is not induced by HCV infection. The possibility of other unknown viral infection has not been excluded, other unknown causes may be responsible for non-alcoholic steatohepatitis, and the possibility of occasional infection of HGBV-C/HGV may remain. Serum HGBV-C/HGV RNA should be successively detected in our case in the future. But the present report suggests that HGBV-C/HGV may be one cause of non-A-E chronic hepatitis and a possible cause of non-alcoholic steatohepatitis. This disease entity is not commonly observed except for cases induced by other specific causes described above. Our case also suggests that there may be cases with HGBV-C/HGV-induced steatohepatitis in patients who have been diagnosed with alcoholic hepatitis. Further study will clarify this suspicion.

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References