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【 CASE REPORT 】

An Autopsy Case of Primary Biliary Cholangitis with Histological Submassive Hepatic Necrosis Caused by Acute Hepatitis E Virus Infection

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Abstract:
A 59-year-old woman who had been diagnosed with cirrhotic primary biliary cholangitis (PBC) 5 years earlier was admitted for severe jaundice (total bilirubin: 30.1 mg/dL). We suspected that her cirrhotic PBC had deteriorated acutely for some reason. Her general condition deteriorated quickly, and she passed away on day 18 of admission. Hepatitis E virus (HEV)-IgA antibodies were positive, and Genotype 3b HEV involvement was confirmed from a blood sample taken on admission. Histopathological findings revealed cirrhosis and submassive loss and necrosis of hepatocytes. Clinicians should consider the possibility of acute HEV infection as a trigger for acute PBC exacerbation.

Key words: primary biliary cholangitis, hepatitis E virus

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Introduction

Primary biliary cholangitis (PBC) is a chronic and slowly progressive cholestatic autoimmune liver disease that is histopathologically characterized by an attack on the small intralobular bile duct by T lymphocytes (1). The precise etiology of PBC remains unknown but is related to genetic susceptibility environmental factors (2-4). Although most patients with PBC are asymptomatic and have a normal life expectancy, clinicians sometimes encounter symptomatic PBC cases with severe jaundice or liver failure as a result of the sustained loss of intralobular bile ducts in spite of ursodeoxycholic acid (UDCA) therapy (5, 6). Patients with concomitant acute liver injury from autoimmune hepatitis show particularly rapid disease progression (7). The acute exacerbation of PBC from hepatitis virus co-infection is rare in the clinical setting.

The hepatitis E virus (HEV) is a quasi-enveloped, single-stranded RNA virus that causes acute or chronic hepatitis (8, 9). With the increase in the number of hepatitis E cases, HEV antibody is now positive in approximately 5% of the general population in Japan (10, 11). Among HEV-associated acute hepatitis cases, 0.5-4% of patients progress to fulminant hepatitis (12), leading to high mortality when intensive care and liver transplantation are unavailable. Rare cases of severe acute HEV hepatitis underlying such chronic liver diseases as alcoholic liver disease, nonalcoholic fatty liver disease, chronic viral hepatitis, and PBC have been documented as well (13-15). No autopsy PBC cases displaying acute exacerbation due to superimposed acute HEV infection have been reported to date.

We herein report the autopsy findings of a PBC case in the cirrhotic stage with histological submassive hepatic necrosis caused by acute HEV infection.

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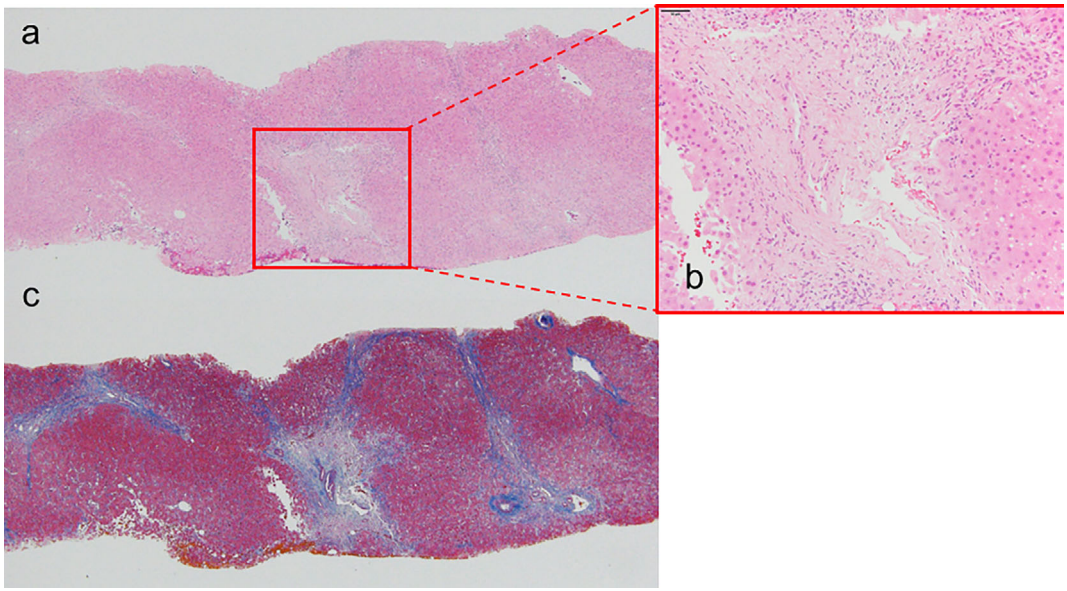

Figure 1. Histological findings of a liver biopsy specimen obtained eight years before admission. a, b) Lymphocyte infiltration in the portal area and severe bile duct loss were observed (Hematoxylin and Eosin staining). c) Azan-Mallory staining showed moderate fibrosis in the liver that was consistent with Nakanuma stage 3 and Scheuer stage III classifications.

Case Report

A 59-year-old woman who had been serologically diagnosed with PBC after the detection of liver dysfunction 26 years earlier was admitted to our hospital for severe jaundice, brown urine, and leg edema. She had suffered from cold-like symptoms and skin jaundice for a month and had received antitussives and antibiotics (levofloxacin 500 mg/day) from her attending physician 2 weeks before admission. She had no symptoms of diarrhea, vomiting, or a history of eating raw meat. She reported no regular ethanol consumption or travel to a foreign country. Although she had been prescribed 600 mg/day UDCA and 400 mg/day bezafibrate for PBC, her medication compliance was extremely poor. She had persistent liver dysfunction [alanine aminotransferase (ALT) at around 100 U/L] and jaundice (total bilirubin at around 3 mg/dL). Eight years before admission, the histological findings of a liver biopsy specimen showed lymphocyte infiltration in the portal area and severe bile duct loss that were consistent with Nakanuma stage 3 (16) and Scheuer stage III (17) classifications (Fig. 1). Three years later, she was clinically diagnosed with cirrhosis-stage PBC. She had also undergone endoscopic gastric varices treatment one year before admission.

On an examination at the time of admission, the patient was 165 cm tall, weighed 68 kg, and had a body mass index of 25.0 kg/m^2. Her vital signs included Glasgow Coma Scale score E4V5M6, body temperature 37.3 °C, and blood pressure 111/54 mmHg. She exhibited conjunctival and systemic jaundice and front chest vascular spider but no flapping tremor. Laboratory tests on admission revealed elevated serum aspartate aminotransferase (78 U/L), ALT (100 U/L), gamma-glutamyl transpeptidase (105 U/L), alkaline phosphatase (702 U/L), total bilirubin (30.1 mg/dL), and Mac-2-binding protein glycan isomer (M2BPGi) (7.4 C·O·I) but a reduced platelet count (7.2×10^4/μL), albumin (2.7 g/dL), and prothrombin time activity (67.7%) (Table 1). Testing for hepatitis A virus antibody (immunoglobulin M), hepatitis B virus surface antigen, and anti-hepatitis C virus (HCV) antibody was negative. Serum antibodies to the Epstein-Barr virus and cytomegalovirus both showed a historical infection pattern. Although serum alpha fetoprotein levels were within normal range, levels of protein induced by vitamin K absence or antagonist II were mildly elevated (152 mAU/mL). Among the autoantibodies tested, fluorescent anti-nuclear antibody (× 640) and anti-mitochondrial M2 antibody (168.1 Index) were positive.

Abdominal ultrasonography showed surface irregularity and a coarse parenchyma echotexture of the liver, suggesting cirrhosis (Fig. 2a). Abdominal contrast-enhanced computed tomography portal phase images showed surface undulation of the liver, collateral circulation around the stomach and splenic hilum, and ascites on the surface of the liver and spleen (Fig. 2b). No evidence suggestive of acute hepatitis, such as periportal collar sign, gallbladder atrophy, or wall thickening, was observed, nor were any space-occupying lesions present in the liver.

Based on the patient's clinical findings, we suspected that her cirrhotic PBC had deteriorated acutely for some reason. Although she did not meet the criteria for acute liver failure, the presence of severe jaundice and ascites indicated the need for liver transplantation. After being refused for this treatment, we provided supportive care, including bed rest, lactulose administration, and albumin replacement. On day 13 of admission, HEV-IgA antibody was found to be posi-

Table 1. Laboratory Findings on Admission.

Blood		Chemistry / Serology		Viral markers	
White blood cell count	5,760 /μL	Total protein	5.9 g/dL	IgM-HAV Ab	(-)
Neutrophils	71.2 %	Albumin	2.7 g/dL	HBs-Ag	0.001 (-) U/mL
Lymphocytes	19.8 %	AST	78 U/L	HBs-Ab	0.4 (-) mIU/mL
Monocytes	5.7 %	ALT	100 U/L	HBc-Ab	0.2 (-) C·O·I
Eosinophils	3.0 %	Total bilirubin	30.14 mg/dL	IgM-HBc Ab	(-)
Basophils	0.3 %	GGT	105 U/L	HCV-Ab	0.1 (-) C·O·I
Red blood cell count	418 $\times 10^4$/μL	ALP	702 U/L	IgM-EBV VCA Ab	<10 (-) ×
Hemoglobin	13.1 g/dL	BUN	12 mg/dL	IgG-EBV VCA Ab	40 (+)
Hematocrit	37.4 %	Creatinine	0.59 mg/dL	EBV EBNA Ab	<10 (-) ×
Platelet count	7.2 $\times 10^4$/μL	NH_3	29 μg/dL	IgM-CMV Ab	0.29 (-) Index
		CRP	1.61 mg/dL	IgG-CMV Ab	133.6 (+) AU/mL
Coagulation		HbA1c	4.2 %	IgA-HEV Ab	(+)
PT	67.7 %	IgM	139 mg/dL		
APTT	29.0 sec	IgA	292 mg/dL	Autoimmune antibodies	
Fibrinogen	192.0 mg/dL	IgG	1,156 mg/dL	FANA	640 ×
D-dimer	2.3 μg/mL	M2BPGi	7.4 C·O·I	AMA2	168.1 Index
				Anti-centromere antibody	(-)
		Tumor markers		LKM-1	(-)
		AFP	7.0 ng/mL	ASMA	(-)
		PIVKA II	152 mAU/mL		

PT: prothrombin time, APTT: activated partial thromboplastin time, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, CRP: C-reactive protein, HbA1c: hemoglobin A1c, M2BPGi: Mac 2-Binding Protein Gylcan Isomer, AFP: alpha fetoprotein, PIVKA II: protein induced by vitamin K absence or antagonist II, FANA: fluorescent antinuclear antibody, AMA2: anti-mitochondrial M2 antibody, LKM-1: anti-liver/kidney microsome type 1 antibody, ASMA: anti-smooth muscle antibody, C·O·I: cut off index

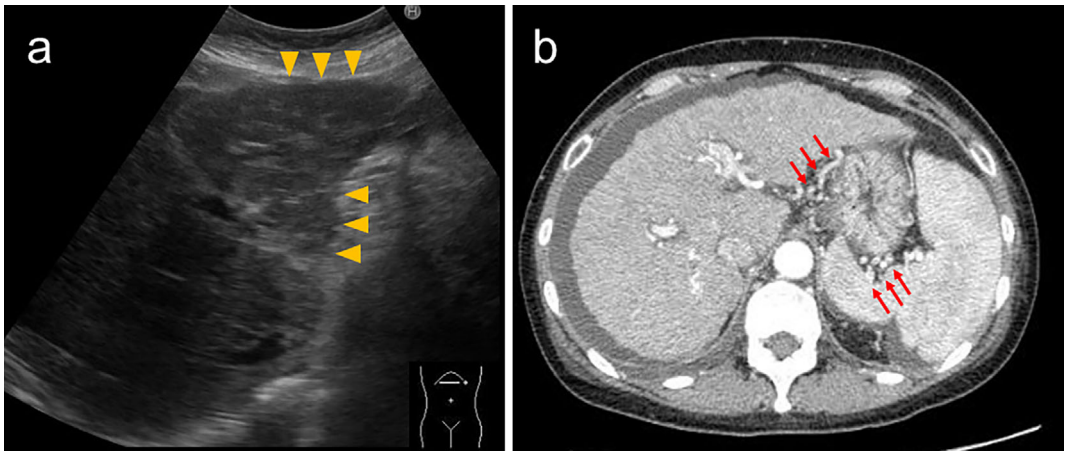

Figure 2. a) Abdominal ultrasonography showed liver surface irregularity (arrowheads) and a coarse parenchyma echotexture. b) Abdominal contrast-enhanced computed tomography portal phase images revealed surface undulation of the liver, collateral circulation around the stomach and splenic hilum (arrows), and ascites on the surface of the liver and spleen.

tive in an admission blood sample, suggesting an association between the exacerbation of her PBC and acute HEV infection. However, the patient's liver atrophy and renal failure were beyond treatment. Her general condition progressively worsened, and she passed away on day 18 of admission. The entire clinical course is summarized in Fig. 3.

A pathological autopsy was performed after obtaining informed consent from her family. Macroscopically, the surface of the liver was atrophied in a nodular manner, indicating complete cirrhosis (Fig. 4a). Microscopic observation of the liver showed severe atrophy with submassive loss and necrosis of hepatocytes from Zone 3 to Zone 2 of the hepatic lobule (Fig. 4b). However, the distribution did not match the centrilobular necrosis typically observed in hepatic ischemia. In some areas, hepatocytes around the central vein were intact (Fig. 4c). In addition, there was no remarkable steatosis inside hepatocytes indicative of hypoxia (Fig. 4c). Based on these findings, hepatic ischemia was not

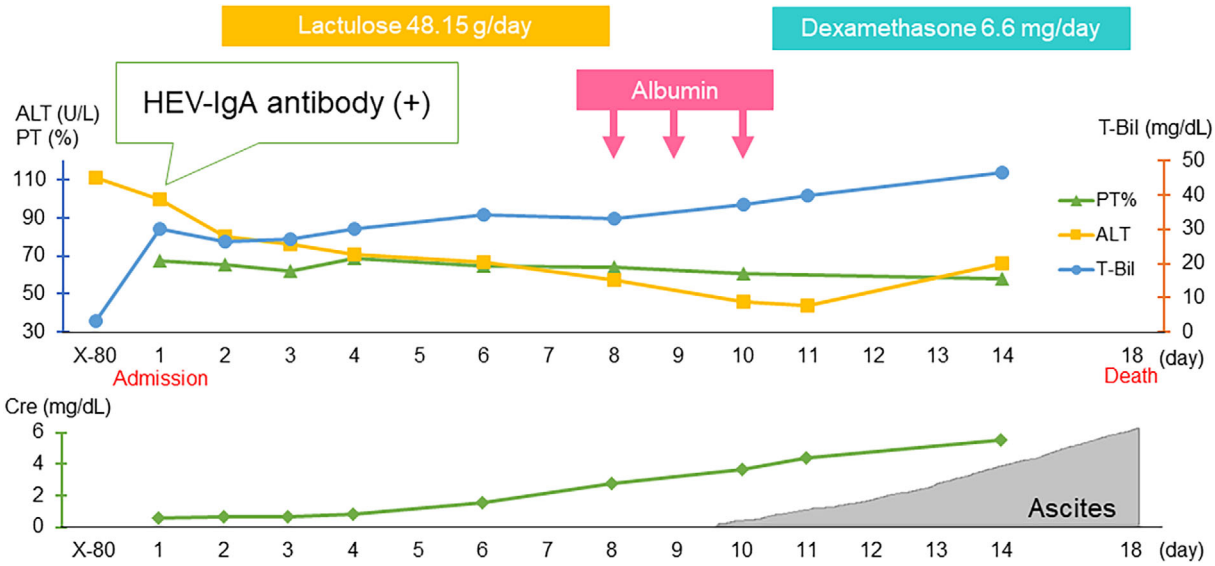

Figure 3. **Clinical course of the patient. ALT: alanine aminotransferase, PT: prothrombin time, T-Bil: Total bilirubin, Cre: Creatinine, HEV: Hepatitis E virus**

considered as the main cause of submassive loss and necrosis in this case. Although cholestasis was prominent in the lobules, inflammatory cell infiltration related to acute hepatitis was not evident (Fig. 4b, c). Regenerated nodules and highly fibrous septum formation were found in the liver, which were histologically consistent with cirrhosis (Fig. 4d). In the portal area, the infiltration of mononuclear cells and disappearance of bile ducts suggested advanced PBC (Fig. 4e). Extensive bile duct loss was confirmed between the interlobular and septal bile duct levels.

HEV antibodies (IgG, IgM, and IgA) were measured by an enzyme-linked immunosorbent assay using frozen serum samples according to a previously described method (18). The testing results are shown in Table 2 along with the corresponding hepatobiliary enzymes at each time point. HEV RNA was detected by the nested reverse-transcription polymerase chain reaction with primers targeting the open reading frame 2 (ORF2) region of the HEV genome (nucleotide position 5944-6355, excluding primer sequences at both ends: M73218), as described previously (19). HEV RNA, which had been negative at the last visit before admission, had become positive at admission along with increases in all classes of HEV antibodies. HEV RNA became undetectable during the deterioration of her condition, although a high antibody titer persisted. We determined her 412-nucleotide ORF2 sequence and constructed a molecular phylogenetic tree according to a previously described method (20) (Fig. 5). The HEV strain (HE-JA19-0280: DDBJ/EMBL/GenBank accession number LC581383) derived from this case belonged to genotype 3 (subgenotype 3b) and was closest to those of patients recovering from acute hepatitis E in Tokyo and Gunma Prefectures of Japan.

Discussion

The present autopsy case displayed histological evidence of submassive necrosis in the liver caused by acute HEV infection. The patient was presumed to have been at the liver cirrhosis stage of PBC but suddenly developed severe jaundice due to superimposed HEV infection and succumbed one month later to hepatic failure. The histopathological findings of severe liver atrophy with submassive loss and necrosis of hepatocytes could not be explained by ischemia only, strongly suggesting the involvement of genotype 3b HEV infection in her histopathological findings.

Although PBC patients generally have a normal life expectancy, they sometimes show rapid disease exacerbation due to concomitant autoimmune hepatitis (7, 21) or simultaneous HCV infection (22) as a trigger of PBC deterioration. One rare instance of PBC with acute HEV infection has been reported to date (15). To our knowledge, however, an autopsy case of rapid deterioration triggered by HEV infection has not yet been described. As the mechanism underlying PBC exacerbation due to viral hepatitis infection remains unknown, the present case may contribute to the body of knowledge concerning this condition.

HEV is one of the most common causes of acute viral hepatitis (23). In addition to contaminated water, raw meat from pigs, boars, and deer is another common infection source (24-26). Recently, the prevalence of HEV cases without clinical symptoms as well as those of unknown transmission route has been increasing (27). The infection route in the present case could not be identified based on our medical interview. According to previous reports, pregnancy, old age, alcohol intake, and fatty liver are host factors associated with the aggravation of HEV, and co-infection with chronic liver disease is related to the risk of liver fail-

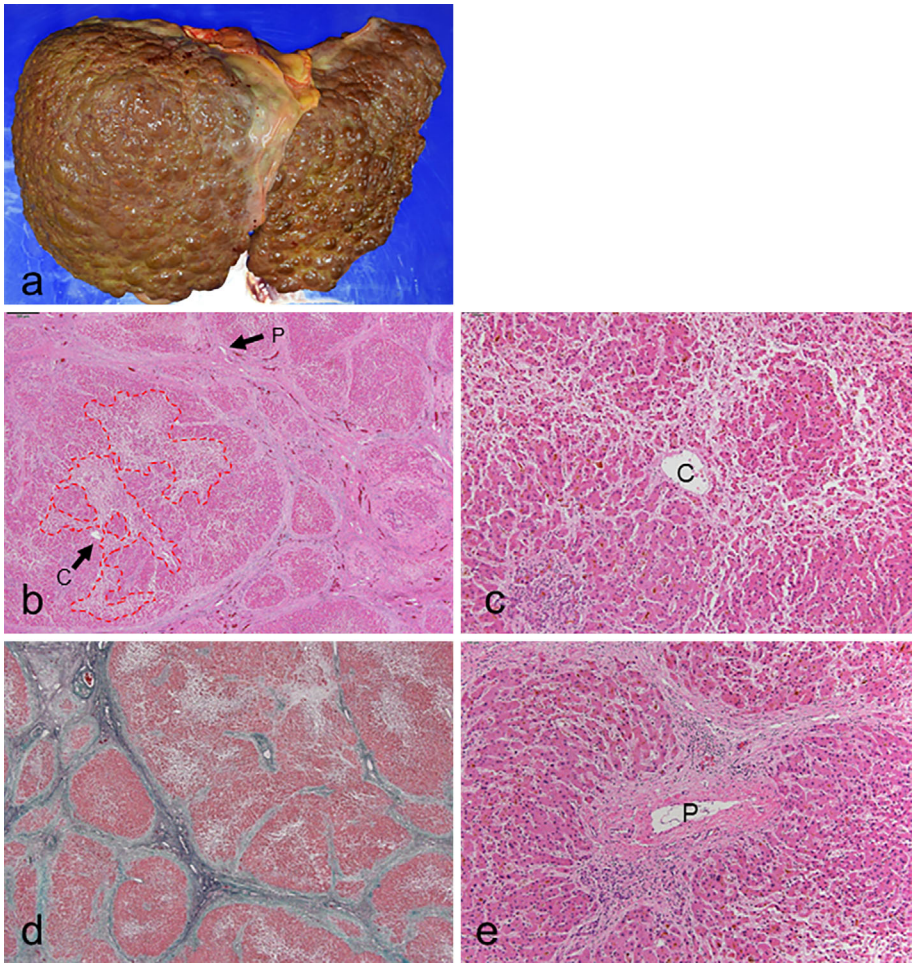

Figure 4. **Autopsy findings. a) Macroscopically, the surface of the liver was atrophied in a nodular manner, indicating complete cirrhosis. b) Severe atrophy with submassive loss and necrosis of hepatocytes (red dotted lines) from Zone 3 to Zone 2 of the hepatic lobule were evident (Hematoxylin and Eosin staining). P: portal vein, C: central vein. c) In some areas, hepatocytes around the central vein were relatively intact, with no steatosis inside hepatocytes suggestive of hypoxia. Cholestasis was prominent in the lobules. Inflammatory cell infiltration indicative of acute hepatitis was not detected. d) On Elastica-Goldner staining, regenerated nodules and highly fibrous septum formation were found in the liver, findings that were histologically consistent with cirrhosis. e) In the portal area, infiltration of mononuclear cells and the disappearance of bile ducts were observed, suggesting advanced PBC. P: portal vein**

Table 2. **Transition of Hepatobiliary Enzymes, HEV Antibodies, and HEV RNA.**

Day	Laboratory data					HEV antibodies						HEV RNA
	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	T-Bil (mg/dL)	IgG (OD_{450})		IgM (OD_{450})		IgA (OD_{450})		
X - 80	137	111	413	940	3.4	0.010	(-)	0.046	(-)	0.020	(-)	Negative
X (admission)	78	100	105	702	30.1	1.661	(+)	>3.000	(+)	2.374	(+)	+: 1.0×10^1 copies/mL
X+1	69	80	87	588	26.6	1.578	(+)	>3.000	(+)	2.346	(+)	+: 1.2×10^1 copies/mL
X+11	43	44	64	568	39.7	1.850	(+)	>3.000	(+)	2.002	(+)	Negative
X+14	61	66	78	631	46.7	1.970	(+)	>3.000	(+)	1.768	(+)	Negative

HEV: hepatitis E virus, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, T-Bil: total bilirubin

ure (13, 14). Regarding the HEV genotype, HEV genotype 4 infection is reportedly associated with aggravation (26, 28, 29). Genotype 3 HEV, as detected in this case, is generally associated with a low tendency of severe clinical manifestations, although a rare case of progressive genotype 3 HEV infection has been reported (28). A mutation of

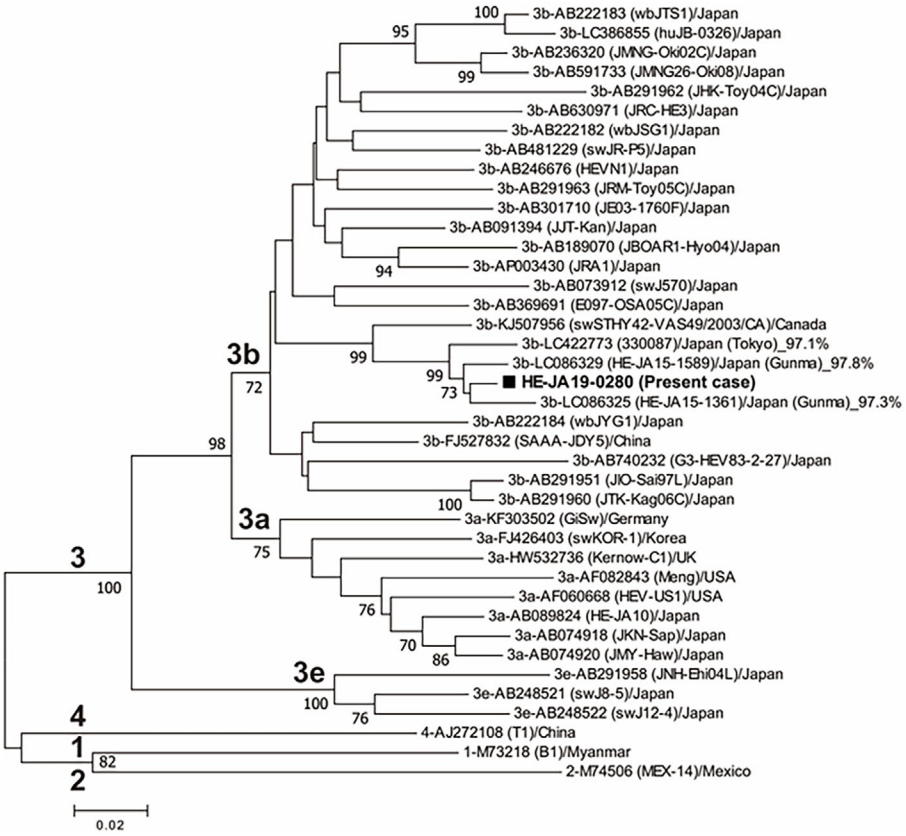

Figure 5. **Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence (412 nucleotides) of the open reading frame 2 region of hepatitis E virus (HEV) isolates. In addition to the HEV isolate (HE-JA19-0280) obtained in the present study (highlighted with a closed box), 33 representative genotype 3 isolates (subgenotype 3a: n=8, subgenotype 3b: n=22, and subgenotype 3e: n=3), whose entire genomic sequence is known, are included in the tree using the prototype HEV sequences of genotype 1 (M73218), genotype 2 (M74506), and genotype 4 (AJ272108) as outgroups. Three HEV isolates sharing the highest identity of 97.1-97.8% with the HE-JA19-0280 isolate are also included. Each reference sequence is shown with the genotype/subgenotype followed by the accession number, isolate name, and country in which it was isolated. The bootstrap values (>70%) are indicated for nodes as the percentage of data among 1,000 resampling procedures. The scale bar is in units of nuceotide substitutions per site.**

V239A in the helicase region of the genotype 3 HEV genome has been associated with HEV infection aggravation (30). That mutant was absent in this case (data not shown). The patient had no other apparent host or viral factors associated with HEV severity. However, Wang et al. reported that the cirrhosis stage itself carries a potential risk of liver failure due to HEV infection, regardless of etiology; they further described the potentiation of the cirrhotic liver upon HEV infection and more aggressive immune- or inflammatory-mediated activation of cell death in a cirrhotic liver status as possible mechanisms of exacerbation (15). The above results suggested that even if patients have no documented risk factors of HEV aggravation, clinicians should bear in mind that HEV infection at a cirrhotic liver disease stage can become severe.

This patient had been diagnosed with PBC 26 years earlier based on serological criteria. Eight years before admission, she was already at an advanced stage of PBC, with Nakanuma stage 3 and Scheuer stage III classifications according to a liver biopsy. An autopsy specimen of the liver revealed obvious cirrhosis, which was consistent with the natural history of PBC as suggested by elevated serum surrogate markers of M2BPGi (31, 32). However, the submassive loss and necrosis of hepatocytes could not solely be explained by the natural PBC history. Inflammatory cell infiltration suggesting acute hepatitis E (33) was not observed, which suggested a post-inflammatory status. Other general clinical features of acute hepatitis, including markedly elevated levels of liver enzymes, were absent as well. Her liver enzymes were only mildly elevated at day X-80, suggesting that asymptomatic hepatitis may have started at that point, followed by further increases in transaminase levels. Thus, one explanation as to why this case showed mild hepatitis was that the hepatitis had occurred after day X-80 and already peaked and ameliorated by the time of admission, such that jaundice represented the major feature. However, no clinical data were available between the patient's last outpatient visit at day X-80 and her hospitalization. Another

hypothesis was that this case had already reached the complete cirrhosis stage, as shown by the pathological findings, which could have induced mild liver enzyme elevations (34).

Patients with hepatitis E sometimes show severe jaundice (35). Although the underlying mechanism is uncertain, HEV has been reported to exhibit not only hepatocytic but also biliary tropism and replicate within bile duct epithelial cells (36, 37). The severe jaundice and cholestasis in this case might have been the result of HEV infection complicating the chronic cholestatic liver disease of PBC. The fact that jaundice was more pronounced than elevated liver enzyme levels may support this notion. Although drug-induced liver injury (levofloxacin 500 mg/day) cannot be ruled out, we suspected that HEV infection likely triggered the exacerbation of PBC due to her awareness of jaundice prior to drug administration and the histological considerations described above.

In conclusion, clinicians should consider the possibility of acute HEV infection as a trigger for the acute exacerbation of PBC. In such cases, it may be necessary to treat the patient with the anticipation of severe aggravation.

The authors state that they have no Conflict of Interest (COI).

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