

Clinical Features of Hepatitis E Virus Infection in Ibaraki, Japan: Autochthonous Hepatitis E and Acute-on-Chronic Liver Failure

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Hepatitis E caused by hepatitis E virus (HEV) is a serious public health concern in developing countries where HEV is mainly transmitted through contaminated water. Recently, in industrialized countries, autochthonous hepatitis E, a porcine zoonosis, has been increasingly recognized. In Japan, the number of national notifications of acute hepatitis E has increased since the introduction of anti-HEV IgA antibody measurement, covered by the national health insurance program, in 2011. In the past three years, we examined five patients of acute hepatitis or acute-on-chronic liver failure caused by HEV infection who presented various clinical courses in the southern area of Ibaraki prefecture in Japan. Of these patients, 78-year-old and 63-year-old male patients presented acute hepatitis E and recovered by only bed rest. The latter patient had a history of consuming grilled or undercooked pork and shellfish prior to the onset of hepatitis E. Among the five patients examined, the infection route was detected only in this patient. Of note, a 65-year-old female patient presented severe hepatitis associated with painless thyroiditis. The patient was diagnosed with probable autoimmune hepatitis and was successfully treated with prednisolone (40 mg/day). Lastly, 58-year-old and 62-year-old male patients, both of whom had a history of diabetes mellitus and alcoholic liver disease, developed acute-on-chronic liver failure, and the latter patient with pre-existing liver cirrhosis died due to liver failure. Thus, patients with clinical HEV infection who display multiple underlying diseases can develop acute-on-chronic liver failure. In conclusion, HEV infection manifests the diverse clinical courses.

Keywords: acute hepatitis; acute-on-chronic liver failure; alcoholic liver disease; autoimmune hepatitis; hepatitis E virus

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Introduction

Hepatitis E virus (HEV) is transmitted via the fecal-oral route and it is an important cause of acute hepatitis worldwide (Purcell and Emerson 2008). HEV infection has traditionally been considered to be a transient form of hepatitis, such as hepatitis A, presenting with fever, fatigue, liver dysfunction and other non-specific symptoms and exhibiting a self-limiting course (Wedemeyer et al. 2012). There are four recognized genotypes (1-4) of HEV that infect humans (Okamoto 2007). HEV strains of genotypes 3 and 4 are known to be zoonotic and autochthonous in both developing and industrialized countries, including European nations, the United States and Japan (Takahashi and Okamoto 2014). Furthermore, HEV infection may occa-

sionally cause severe liver dysfunction and fulminant or chronic hepatitis in some patients (Suzuki et al. 2002; Kamar et al. 2012).

In Japan, sporadic acute or fulminant hepatitis E have been reported in recent years (The National Institute of Infectious Diseases: <http://www.nih.go.jp/niid/ja/idwr.html>). Autochthonous HEV strains obtained from humans and animals in Japan belong to genotype 3 or 4, and genotype 3 HEV strains indigenous to Japan have been provisionally classified into three subgenotypes: 3b (3jp), 3a (3us) and 3e (3sp), where “jp” stands for Japan-type, “us” for US-type and “sp” for Spanish (European) type (Okamoto et al. 2003; Takahashi et al. 2003; Lu et al. 2006).

Additionally, the number of national notifications of

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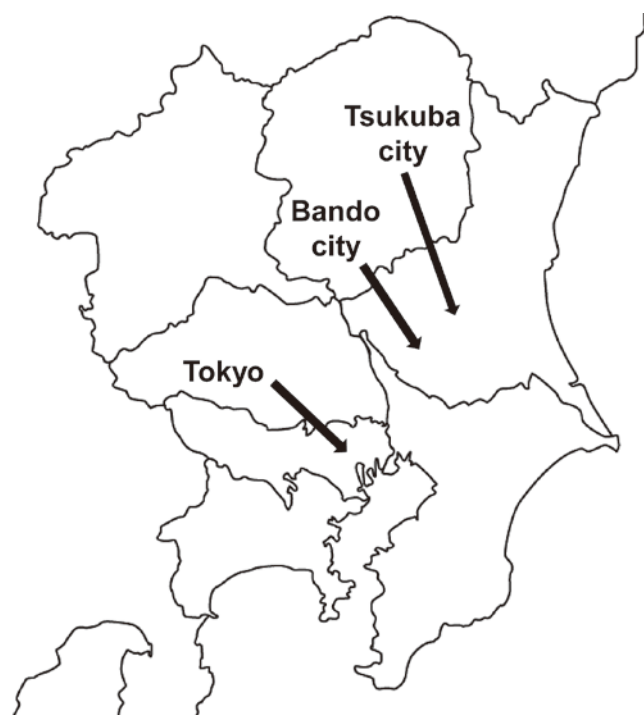


Fig. 1. The location of the patients.
Map of Ibaraki prefecture showing Tsukuba and Bando cities where our patients with acute hepatitis E lived.

acute hepatitis E has increased since the introduction of anti-HEV IgA antibody measurement, covered by the national health insurance program, in 2011 (The National Institute of Infectious Diseases: <http://www.nih.go.jp/niid/ja/idwr.html>), and the presence of domestic HEV as a causative agent of acute hepatitis is increasingly being recognized. In the past three years, we experienced five patients with hepatitis E, including a patient with suspected autoimmune hepatitis and the patients with acute-on-chronic liver failure with underlying diseases, such as diabetes mellitus, chronic renal failure, and alcoholic liver disease, in the southern area of Ibaraki prefecture, Japan (Fig. 1).

Patients

The clinical features of the five patients with acute hepatitis or acute-on-chronic liver failure caused by HEV are briefly presented in Table 1. Patients 1 and 2 involved a mild form of acute hepatitis E that improved spontaneously and ran a self-limiting course without sequelae. Meanwhile, the remaining three patients developed acute liver failure without overt hepatic encephalopathy (a severe form hepatitis E: Patient 4) or with hepatic encephalopathy (acute-on-chronic liver failure: Patients 3 and 5) based on the criteria for these diseases in Japan (Sugawara et al. 2012; Mochida et al. 2014). The patients in Patients 3 and 4 survived, while the patient in Patient 5 died. The clinical

Table 1. Clinical features of patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age/Gender	78/M	63/M	58/M	65/F	62/M
Onset	Apr. 2013	May. 2013	Feb. 2010	Dec. 2011	Jan. 2012
Address	Tsukuba city	Tsukuba city	Tsukuba city	Bando city	Bando city
Previous disease	Hypertension, Hyperuricemia	Diabetes	Diabetes, Angina	Painless, thyroiditis	Diabetes, Chronic renal failure, Liver cirrhosis
Alcohol intake	70 g/day	30 g/day	140 g/day	–	120 g/day
Diet/foreign travel history	–/–	+/-	–/–	–/–	–/–
Initial symptom	Jaundice	General fatigue	General fatigue	Fever, Jaundice	Fever, General fatigue
Peak value of AST (IU/L)	1,175	849	1,306	6,150	5,478
Peak value of ALT (IU/L)	957	1,596	1,141	4,958	2,235
Peak value of total bilirubin (mg/dL)	7.5	1.0	19.8	19.2	28.9
Minimum PT (%)	99.0	98.6	12.8	31.1	36.6
Genotype	3jp	3jp	NA‡	NA	NA
Liver biopsy	–	+	–	+	+
Treatment	MAG†	MAG	MAG	Steroid, Hyper-alimentation	Plasmapheresis, Dialysis
Disease progression	Self-limited	Self-limited	Acute-on-chronic liver failure	Severe hepatitis	Acute-on-chronic liver failure
Outcome	Survive	Survive	Survive	Survive	Death

†MAG, monoammonium glycyrrhizinate.

‡NA, not available.

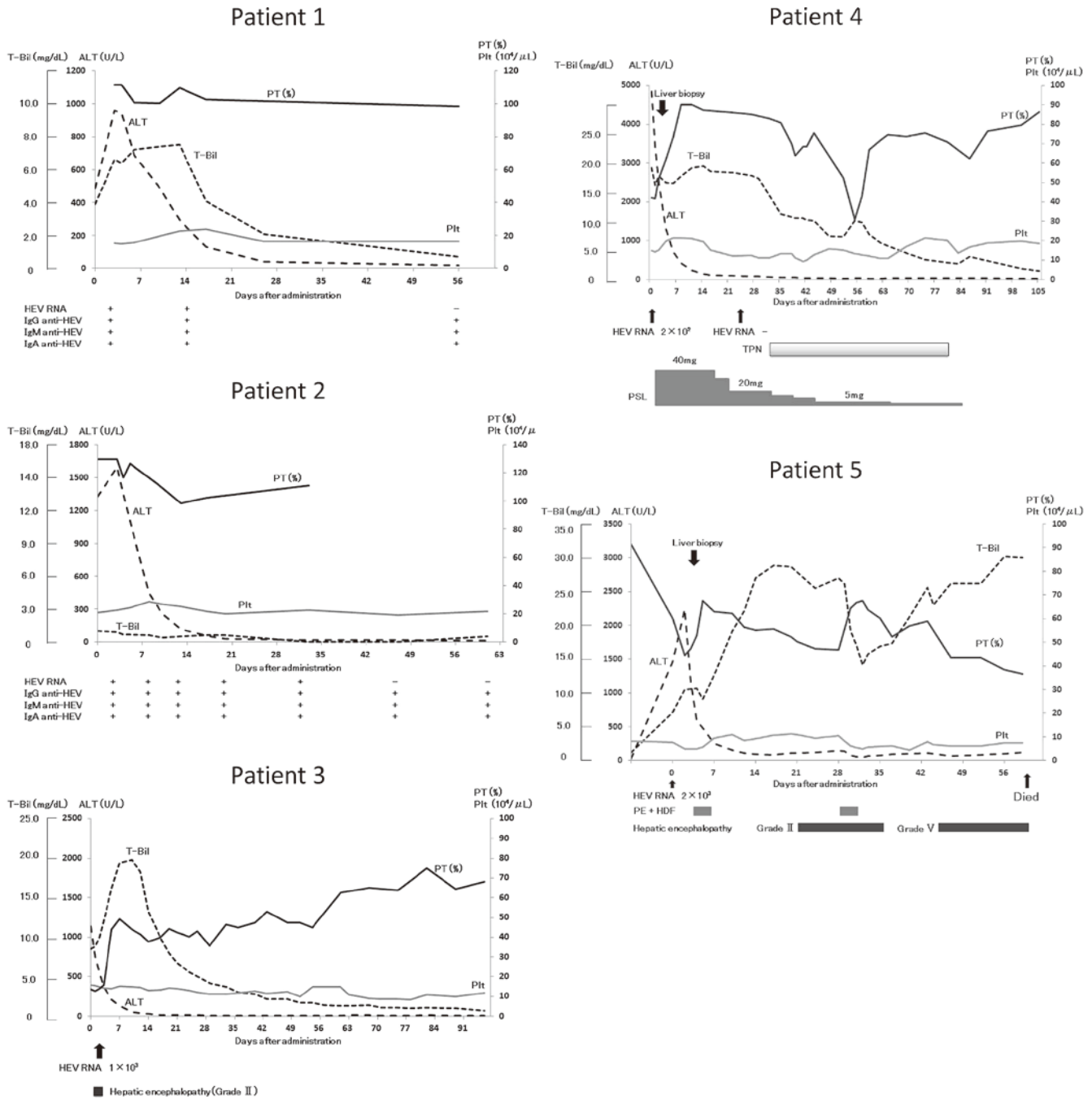


Fig. 2. Clinical courses of the five patients.

Patients 1 and 2 involved a mild form of self-limiting hepatitis. Patient 3 contracted fulminant hepatitis E, but survived. Patient 4 had severe acute hepatitis E and was given steroids according to the pretreatment diagnosis of autoimmune hepatitis. Patient 5 presented with fulminant hepatitis E that progressed to liver failure and was treated with plasma-pheresis and dialysis.

T-Bil, total bilirubin; ALT, alanine aminotransferase; PT (%), prothrombin time %; Plt, platelet; TPN, total parental nutrition; PSL, prednisolone; PE, plasma exchange; HDF, hemodiafiltration.

courses of the patients are shown in Fig. 2.

Results

Patients 1 and 2

With a chief complaint of yellow urine or general fatigue, a 78-year-old and 63-year-old male living in

Tsukuba city were each admitted to our hospital. Laboratory tests on admission revealed significantly elevated levels of liver enzymes with or without jaundice. Serological and molecular markers of hepatitis A, B and C viruses (HAV, HBV and HCV) and other markers of hepatitis-causing viruses, such as Epstein-Barr virus (EBV) and

cytomegalovirus (CMV), were all negative, whereas anti-HEV IgA antibodies were positive according to an enzyme-linked immunosorbent assay (ELISA) with IMMUNIS[®] IgA anti-HEV EIA (Institute of Immunology Co., Ltd., Tokyo, Japan). The presence of IgG and IgM classes of anti-HEV antibodies (Takahashi et al. 2005) and HEV RNA (Mizuo et al. 2002) in serum was subsequently tested at the Division of Virology, Department of Infection and Immunity, Jichi Medical University School of Medicine, and all HEV markers were found to be positive (Fig. 2). Other causes of acute liver dysfunction, such as drugs or autoimmunity, were also excluded. Therefore, these two patients were finally diagnosed with acute hepatitis E. Their symptoms and liver dysfunction improved with bed rest and the administration of glycyrrhizin [stronger Neo-Minophagen C (SNMC); Minophagen Pharmaceutical Co., LTD., Tokyo, Japan]. Patients 1 and 2 became negative for HEV RNA in the serum on days 53 and 47 of the illness, respectively. Since these two HEV infections occurred at almost the

same time in neighboring areas within Tsukuba city, we analyzed the nucleotide sequence of each HEV isolate within the ORF2 region. Contrary to our expectations, the HEV isolates in these patients shared only 89.2% nucleotide sequence identity and segregated into separate clusters of subgenotype 3b/3jp within genotype 3 (Fig. 3) in a phylogenetic tree constructed according to the neighbor-joining method (Tamura et al. 2013). The nucleotide sequence data determined in this study were assigned DDBJ/EMBL/GenBank accession numbers AB986279-AB986280.

Patient 3

The patient was a 58-year-old male living in Tsukuba city with complaints of general fatigue and a disturbance of consciousness lasting for a few days before admission. Laboratory tests performed on admission revealed elevated levels of liver enzymes and bilirubinemia and a significantly decreased prothrombin time (PT) %. The serum HEV RNA titer was 1×10^3 copies/ml. The CT scan on

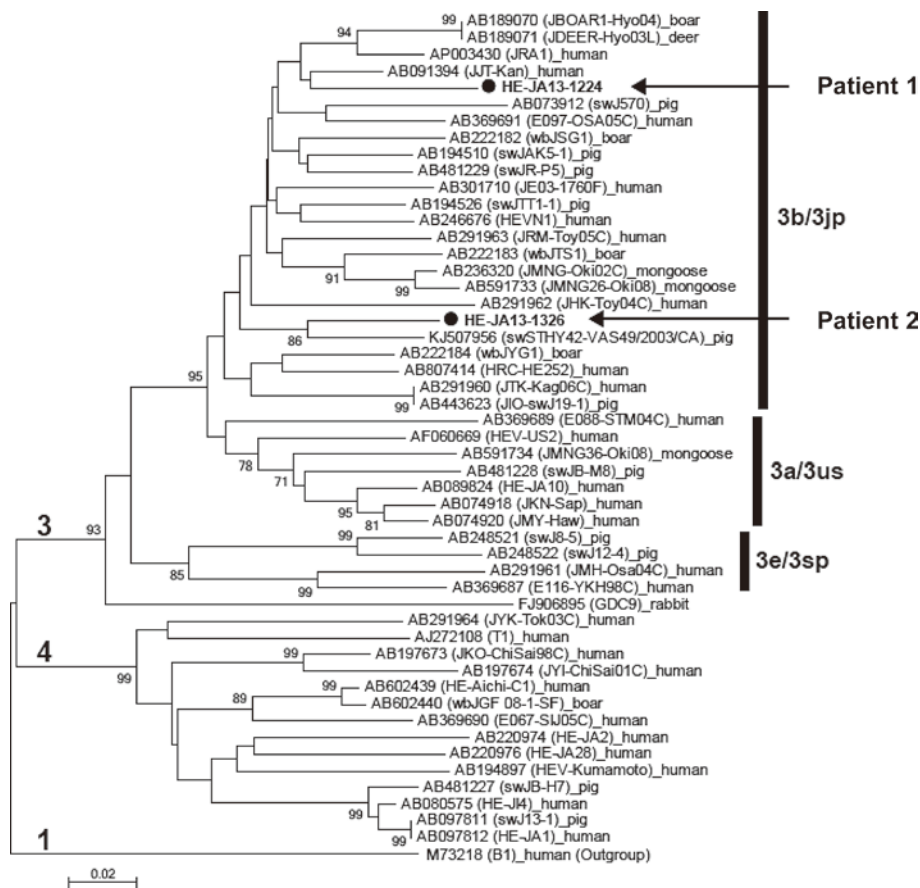


Fig. 3. Phylogenetic tree of HEV recovered from Patients 1 and 2.

Phylogenetic tree constructed according to the neighbor-joining method based on the 50 partial nucleotide sequences of the ORF2 region (412 nt), using a prototype genotype 1 HEV (M73218) as an outgroup. The HE-JA13-1224 (Patient 1) and HE-JA13-1326 (Patient 2) isolates obtained in the present study are indicated in bold type and highlighted with closed circles. The reference sequences are shown with accession numbers followed by the isolate name in parenthesis, and the name of the animal species from which it was isolated. Japan-indigenous genotype 3 isolates are divided into three subgenotypes: 3a/3us, 3b/3jp and 3e/3sp (Okamoto et al. 2003; Takahashi et al. 2003; Lu et al. 2006). The bootstrap values (> 70%) are indicated for the nodes as a percentage of the data for 1,000 re-sampling procedures. The scale bar is in units of nucleotide substitutions per site.

admission showed the fatty liver change and liver cirrhosis. Despite the presence of severe liver dysfunction, the patient recovered spontaneously following bed rest and the administration of glycyrrhizin (Fig. 2).

Patient 4

A 65-year-old female living in Bando city (Fig. 1) presented to our hospital with general fatigue and yellow urine. In addition to severe liver dysfunction, the immunoglobulin G (IgG) level was 2,020 mg/dl ($< 1,600$) and serological tests for autoimmune disease revealed positive anti-thyroglobulin antibodies (333.6 IU/mL; < 28). There were no viral markers of acute HAV, HBV, HCV, EBV or CMV infection or epidemiological history suggesting HEV infection. The patient's pretreatment International Autoimmune Hepatitis Group (IAIHG) score (Alvarez et al. 1999) was 11 [female (+2), alkaline phosphatase (ALP)/aspartate aminotransferase (AST) ratio < 1.5 (+2), IgG = $1.26 \times$ normal upper limit (+1), negative for antimicrosomal antibodies (AMA), alcohol intake < 25 g/day (+3), hepatotoxic drugs negative (+1), thyroiditis (+2)], and she was diagnosed with probable autoimmune hepatitis complicated with painless thyroiditis. Immunosuppressive therapy with prednisolone (40 mg/day) was initiated, and, after one week of therapy, a significant improvement was noted in the laboratory test

results. A liver biopsy performed on admission showed the infiltration of mononuclear cells and submassive hepatic necrosis without interface hepatitis, compatible with the findings of autoimmune hepatitis (Fig. 4A and B). Meanwhile, serological and molecular tests for HEV infection in the serum conducted on admission indicated ongoing HEV infection. Despite the improvement in the patient's liver function, she experienced continued appetite loss and required total parental nutrition for several weeks. The dose of prednisolone was gradually tapered, and she subsequently recovered (Fig. 2). After the tapering of prednisolone, hepatitis did not relapse.

Patient 5

The patient was a 62-year-old male living in Bando city (Fig. 1) who had been treated for diabetes mellitus, chronic renal dysfunction and non-B, non-C liver cirrhosis at a neighboring hospital. On emergency admission, he presented with fever, fatigue and severe liver dysfunction according to blood tests. The CT scan on admission showed multiple liver cysts and liver cirrhosis. Serum HEV RNA was positive, and a liver biopsy showed submassive hepatic necrosis and cirrhosis (Fig. 4C and D). Although plasma exchange and hemodialysis were performed twice during hospitalization, the serum bilirubin level increased,

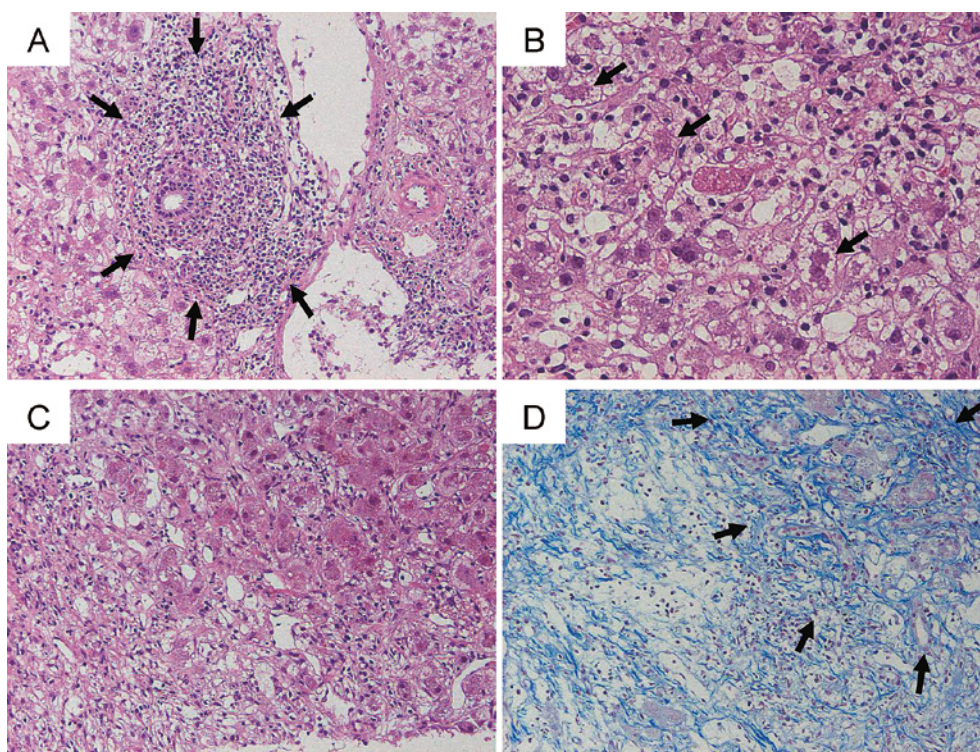


Fig. 4. Liver biopsy findings in Patients 4 and 5.

(A) Portal area in Patient 4: There were findings of moderate infiltration of mononuclear cells (➡) without interface hepatitis ($\times 40$, H&E stain). (B) Lobular area in Patient 4: There were findings of submassive necrosis; most hepatic cells were degenerative and necrotic. Parenchymal cells with vacuolar and eosinophilic sloughing (➡) were associated with mononuclear infiltration ($\times 200$, H&E stain). (C) Liver cirrhosis and submassive necrosis of hepatic cells was observed in Patient 5 ($\times 100$, H&E stain). (D) Hepatic cell necrosis with slight lymphocyte infiltration was scattered in the pseudolobules and fibrosing areas (➡) in Patient 5 ($\times 100$, Masson trichrome stain).

and the patient went into a coma as a result of hepatic encephalopathy and subsequently died on day 65 after admission.

Discussion

HEV is transmitted enterically and causes acute self-limiting or fulminant hepatitis, which is endemic in many developing countries in Asia, Africa and Latin America, where sanitation conditions are suboptimal (Purcell and Emerson 2008; Wedemeyer et al. 2012; Takahashi and Okamoto 2014). HEVs capable of infecting humans are classified into four major genotypes (genotypes 1-4) according to their sequence. Genotypes 1 and 2 are restricted to humans and transmitted via contaminated water in developing countries. Genotypes 3 and 4 are zoonotic and infect humans as well as pigs and other mammalian species and are responsible for autochthonous hepatitis E in both developing and developed countries (Okamoto 2007; Takahashi and Okamoto 2014).

Since 2001, sporadic acute hepatitis caused by autochthonous HEV has been reported with increasing frequency in Japan (Takahashi et al. 2001; Mizuo et al. 2002; Okamoto et al. 2003). Sporadic or cluster patients of clinical HEV infection in individuals with a recent history of consuming meat and/or the internal organs of pigs or wild animals (boars and deer) have also been reported (Tei et al. 2003; Yazaki et al. 2003; Tamada et al. 2004; Li et al. 2005). Furthermore, public attention to the potential for HEV culminating in fatal patients of severe acute or fulminant hepatitis E has increased (Suzuki et al. 2002; Ohnishi et al. 2003; Mizuo et al. 2005; Inoue et al. 2006). Hence, hepatitis E is a topic of interest that has promoted rapid advances in research regarding the diagnosis and epidemiology of HEV infection. The annual number of reported hepatitis E patients in Japan was approximately 54 from 2004 to 2011 (The National Institute of Infectious Diseases: <http://www.nih.go.jp/niid/ja/idwr.html>). In 2011, the national health insurance program in Japan started to cover the measurement of anti-HEV IgA antibodies for diagnosis. Since then, the number of reported hepatitis E patients has been increasing, with 121 patients in 2012 and 126 in 2013 (The National Institute of Infectious Diseases: <http://www.nih.go.jp/niid/ja/idwr.html>). The largest number of hepatitis E patients has long been reported in Hokkaido, the northernmost prefecture in Japan. However, in recent years, the number of hepatitis E patients has been increasing in the Kanto area, as represented by Tokyo. In accordance with the findings in Tokyo, the number of hepatitis E patients in Ibaraki prefecture, which is located 60 km northeast of Tokyo in the northern Kanto area, has been increasing slowly but steadily. Of note, however, there are currently no patient reports of hepatitis E in the southern area of Ibaraki prefecture.

In the current Patients 1 and 2, the liver enzyme levels were significantly elevated, although they improved without intensive care. The patients were subsequently diagnosed

with a mild form self-limiting hepatitis E. Swine HEV isolates have been frequently identified in domestic pigs in Japan (Takahashi et al. 2003). The most likely cause of acute hepatitis E in Japan is thought to be the consumption of meat or internal organs from contaminated pigs, and a positive correlation has been recognized between the prevalence of anti-HEV IgG and expenditure for pork in each prefecture (Takahashi and Okamoto 2014). It is likely that foods such as shellfish also act as vehicles for HEV transmission (Koizumi et al. 2004; Crossan et al. 2012; Scobie and Dalton 2013). Among our patients, only Patient 2 had a history of consuming grilled or undercooked pig liver and/or intestines as well as shellfish prior to the onset of hepatitis E. Although Patients 1 and 2 lived in the same city, the HEV isolates obtained from these patients shared only 89.2% nucleotide sequence identity. Accordingly, the patients were sporadically infected with different HEV isolates. Although domestic HEV infection is thought to be mostly zoonotic in developed countries, identifying definitive food sources in individuals is often difficult.

In Patient 4, we made a pretreatment diagnosis of probable autoimmune hepatitis based on the patient's high IAIHG score (Alvarez et al. 1999) of 11 and complications with painless thyroiditis. Vieira et al. (2013) reported a hepatitis E patient with concomitant signs of autoimmunity in which the patient showed positive findings for autoimmune antibodies, including anti-thyroid antibodies. There are few other reports of hepatitis E complicated by autoimmune thyroiditis (Vento and Cainelli 2004; Pischke et al. 2014). However, patients with autoimmune hepatitis have been reported to be more likely to be positive for anti-HEV antibodies (Vento and Cainelli 2004; Pischke et al. 2014). HEV may therefore be a trigger for autoimmune mechanisms, such as HAV (Vento and Cainelli 2004), and patients with autoimmune hepatitis may present with apparent HEV infection as well as other chronic liver diseases (Vento and Cainelli 2004; Pischke et al. 2014). Hence, HEV infection should be ruled out in patients with autoimmune hepatitis, exhibiting exacerbation of liver dysfunction.

In Patients 3 and 5, hepatic encephalopathy and a prolonged prothrombin time under 40% met the diagnostic criteria for acute-on-chronic liver failure. Patient 5, with pre-existing comorbid liver cirrhosis, finally died due to liver failure. Both patients had a history of diabetes mellitus and a large amount of alcohol consumption for approximately 30 years, and the CT imaging on admission revealed the fatty liver change and liver cirrhosis. Several previous studies have reported that excessive alcohol abuse and chronic liver disease are more frequent among patients with severe liver dysfunction or jaundice resulting from HEV infection (Kumar Acharya et al. 2007; Péron et al. 2007; Radha Krishna et al. 2009; Xu et al. 2012). Meanwhile, other studies have described the efficacy of ribavirin monotherapy against acute HEV infection in patients with a pre-existing chronic liver disease (Gerolami et al. 2011; Péron et al. 2011), suggesting that treatment with ribavirin should

have been considered in Patient 5, although this drug is not covered by national health insurance in Japan.

Our present study has two limitations. First, we could not detect the infectious route of HEV in Patients 1, 3, 4 and 5. In a previous study in Japan, the most likely transmitting route of HEV was reported as the consumption of pig liver or intestine in the Kanto area including Ibaraki prefecture (Takahashi and Okamoto 2014). Second, the HEV genotype was unknown in Patients 3-5, which involved severe hepatitis or acute-on-chronic liver failure, due to the unavailability of stored serum samples in these three patients. Though over 80% of past hepatitis E patients that occurred in the Kanto area were caused by genotype 3 (Takahashi and Okamoto 2014), genotype 4 is well known to be associated with more aggressive hepatitis and fulminant hepatitis than that of genotype 3 (Mizuo et al. 2005; Ohnishi et al. 2006). It was possible that our severe patients included hepatitis E caused by genotype 4.

In conclusion, we herein experienced five patients of acute hepatitis E that presented with various clinical courses from mild hepatitis to acute-on-chronic liver failure. The present study suggests that patients with clinical HEV infection who display multiple underlying diseases, such as chronic renal failure, diabetes mellitus and alcoholic liver injury, may develop acute-on-chronic liver failure.

Conflict of Interest

The authors declare no conflict of interest.

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