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Case Report

A Patient with Clinical Features of Acute Hepatitis E Viral Infection and Autoimmune Hepatitis

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NAGASAKI, F., UENO, Y., MANO, Y., IGARASHI, T., YAHAGI, K., NIITSUMA, H., OKAMOTO, H. and SHIMOSEGAWA, T. *A Patient with Clinical Features of Acute Hepatitis E Viral Infection and Autoimmune Hepatitis*. Tohoku J. Exp. Med., 2005, **206** (2), 173-179 —— Hepatitis E virus (HEV) is one of the major causative agents of acute hepatitis in many developing countries. Recent intensive examination has revealed the existence of non-imported cases in industrialized countries. The patient was a 25-year-old Japanese female with acute hepatitis. Laboratory test demonstrated positive anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) and high level of serum immunoglobulin G (IgG). The patient was negative for serum markers of hepatitis A, B or C virus infection. She demonstrated a clinical course similar to severe autoimmune hepatitis, including response to prednisolone therapy. After a few years, with the availability of tests for the serum antibodies to HEV, we examined the frozen stocked sera of the patient and found her exact diagnosis was acute hepatitis E. Although we could not detect HEV-RNA, which is positive only in limited period of acute phase, serum IgA and IgG antibodies to HEV were positive and the titer of IgA and IgG antibodies were declined with the time course. In conclusion, we must take into consideration of HEV infection for the diagnosis of acute cryptogenic hepatitis including autoimmune hepatitis. Further studies are feasible to understand the pathogenesis of liver injuries induced by HEV infections. ———— acute hepatitis; hepatitis E virus; autoimmune hepatitis; viral hepatitis

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Hepatitis E virus (HEV) that has a single and positive-strand RNA genome of approximately 7,200 nucleotides is one of the major causative agents of acute hepatitis in many developing countries in Asia, Africa and Central America. HEV is transmitted via fecal-oral route (Balayan et al. 1983; Reyes et al. 1990; Hino et al. 1991; Tam et al. 1991; Zaaijer et al. 1993; Scharschmidt 1995; Purcell and Emerson 2001; Emerson and Purcell 2003). However, the availability of recent intensive examination has revealed the existences of some non-imported cases even in industrialized

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countries. Thus, it is now believed that the virus is much more diverse and widespread than expected. Up to now, 4 major genotypes have been identified (Schlauder and Mushahwar 2001).

HEV infection has scarcely been reported in Japan, and most of the cases observed so far have been recognized as imported ones (Hino et al. 1991; Ishikawa et al. 1995). However, more recently, several sporadic cases without history of traveling to endemic area of HEV have been reported (Takahashi et al. 2001; Suzuki et al. 2002; Takahashi et al. 2002a; Ohnishi et al. 2003; Yazaki et al. 2003).

In this report, we present a patient with features of acute hepatitis E infection, who had never been abroad and demonstrated a clinical course similar to severe autoimmune hepatitis (AIH).

Patient

The patient was a 25-year-old Japanese housewife. Her previous medical history revealed nothing particular events such as blood transfusion or operations except for artificial abortion. She had an occasional alcohol intake, but no history of traveling abroad and no contact with foreign travelers. She did not receive any drug medication or intravenous injection.

Present illness

She felt deteriorating abdominal distension at December 9th, 2000. Her local physician pointed out the presence of ascites by abdominal ultrasound sonography (US). Next day, she was referred to a gynecologist, although there was not any gynecological abnormality. She also felt abdominal pain at December 14th, and visited another local hospital. Laboratory tests revealed anemia (Hemoglobin [Hb] 8.0 mg/100 ml), thrombocytopenia (Platelet [Plt] $71 \times 10^3/\mu$l), elevated liver enzymes (asparate aminotranferase [AST] 222 IU/l, alanine aminotransferase [ALT] 230 IU/l) and elevated total bilirubin (T.Bil) 6.6 mg/100 ml. Moreover, splenomegaly was detected by computed tomography (CT) scan. Therefore, bone marrow aspiration was performed, and the presence of hematological disease was ruled out. Thereafter, she was referred to our hospital, and admitted on the December 15th, 2000.

Physical findings at administration

Physical examination revealed: height 153 cm; body weight 52.9 kg; blood pressure 124/80 mmHg; body temperature 37.7°C; and clear consciousness. The bulber conjunctiva was icteric. No peripheral edema, vascular spider, palmar erythema, and flapping tremor were observed.

Laboratory findings

She was admitted on the late Friday night, thus we could not perform routine laboratory tests. The result of initial tests revealed liver dysfunction, coagulopathy, anemia and thrombocytopenia (Table 1). Her routine laboratory test on the 4th hospital day is shown on the Table 2. Of note, anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA) were positive, although anti-mitochondria antibody (AMA) was negative. Serum markers of hepatitis A, B or C virus were all negative.

Clinical course

Prior to obtaining entire laboratory tests, we temporally diagnosed that the patient had an acute hepatic failure, and started to transfuse fresh frozen plasma and albumin. Abdominal CT did not show space occupying lesions or the presence of chronic liver disease except for mild splenomegaly, huge amount of ascites and pleural effusion.

On the 7th hospital day, we serologically

TABLE 1. *Laboratory findings on admission*

WBC	9,200/μl	RBC	$241 \times 10^4/\mu$l
Hb	8.5 g/100 ml	Plt	$88 \times 10^3/\mu$l
T.Bil	9.3 mg/100 ml	AST	230 IU/l
ALT	235 IU/l	ALP	409 IU/l
LDH	423 IU/l		
CRP	0.4 mg/100 ml	BUN	10 mg/100 ml
Cr	0.5 mg/100 ml	PT	31%

WBC, white blood cell; CRP, C-reactive protein; Cr, Creatine; RBC, red blood cell; ALP, alkaline phosphatase; BUN, blood urea nitrogen; PT, prothrombin time.

TABLE 2. *Laboratory findings on 4th hospital day*

WBC	5,100 /μl	RBC	$206 \times 10^4/\mu$l
Hb	7.0 g/100 ml	Plt	60×10^3 /μl
PT	43%	Hepaplastin test	43%
T.Bil	6.4 mg/100 ml	Direct Bil	3.6 mg/100 ml
AST	111 IU/l	ALT	116 IU/l
ALP	318 IU/l	GGTP	25 IU/l
LDH	368 IU/l		
CRP	4.1 mg/100 ml		
BUN	11 mg/100 ml	Cr	0.5 mg/100 ml
Total protein	7.1 g/100 ml	Albumin	2.8 g/100 ml
IgM-HAAb	negative		
HBs-Ag	negative	IgM-HBcAb	negative
HCV-Ab	negative		
HIV-Ab	negative		
ANA	× 80	ASMA	≧ ×160
AMA	negative		
IgG	3,585 mg/100 ml	IgA	294 mg/100 ml
IgM	511 mg/100 ml		

WBC, white blood cell; PT, prothrombin time; AST, asparate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehidrogenase; CRP, C-reactive protein; BUN, blood urea nitrogen; ANA, anti nuclear antibody; AMA, anti mitochondria antibody; RBC, red blood cell; Plt, platelet; ALT, alanine aminotransferase; GGTP, gamma glutamil transpeptidase; Cr, creatine; ASMA, anti-smooth muscle antibody.

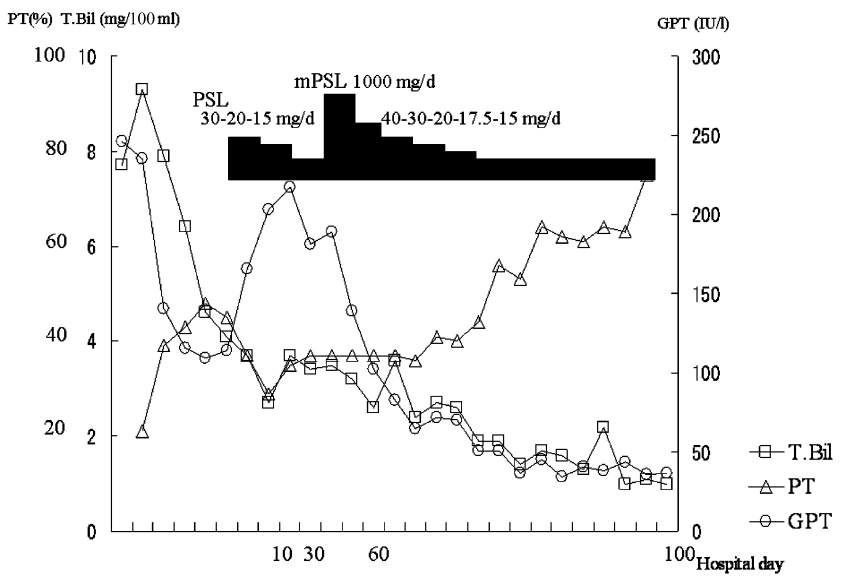

Fig. 1. Clinical course of the patient. The patient recovered after administration of corticosteroid.

made diagnosis to the case as acute onset type AIH, and started the administration of prednisone (PSL) 30 mg/day (Fig. 1). However, she showed minimal response to the therapy. Therefore, we administrated methyl-PSL 1g/day for three days, which was followed by PSL 40 mg/day and tapered over weekly. Her general condition as well as clinical data had improved, and finally she discharged on March 26th, 2001. During this admission, the unexpected illness of her family prevented further extensive examinations including liver biopsy.

Finally the patient underwent the laparoscopic liver biopsy at 8 months after the onset, when PSL had been already withdrawn and laboratory tests showed normal liver function. Pathological findings of the specimens showed inflammatory changes in the portal vein area, and the presence of bridging necrosis. It was compatible with the recovery phase of acute severe hepatitis (Figs. 2 and 3).

We administrated PSL for 8 months according to these data, and could withdraw it ultimately. It has been 1.5 years since the withdrawal of PSL, and her transaminase, serum IgG level and ANA are all within normal limit without any medication. At the last visit to our department (Oct 15th, 2002), her laboratory examination demonstrated normal liver function (ALT 15 IU/l, AST 15 IU/l), normal serum immunoglobulins

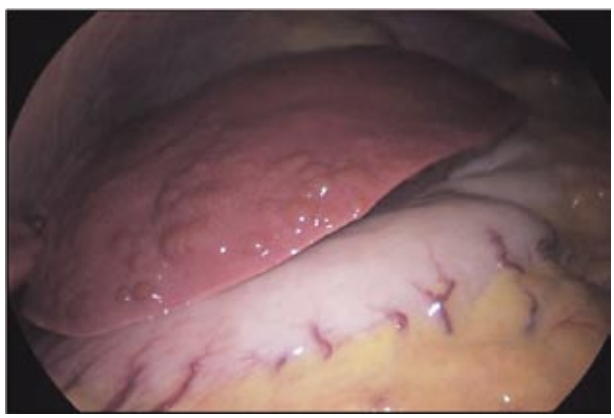

Fig. 2. Macroscopic pathological findings. Laparoscopy was performed on August 8th, 2001. Uneven liver surface due to severe and massive inflammation was apparent. This morphological view clearly ruled out the presence of cirrhosis.

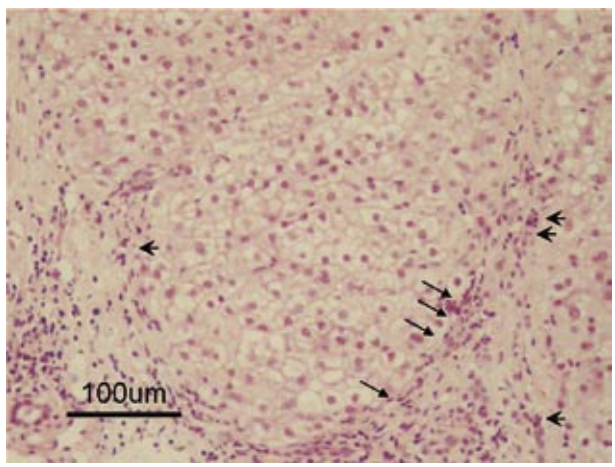

Fig. 3. Microscopic pathological findings. The specimen did not demonstrate classical features of AIH, such as infiltration of plasma cells (arrow heads) or presence of interface hepatitis (arrows). This biopsy was compatible with the recovery phase of severe acute inflammation. (H&E, × 20)

TABLE 3. *Titer of anti-HEV assay (ELISA)*

Date	IgM-HEV	IgG-HEV	IgA-HEV	HEV-RNA
2000.12.15	0.253 (–)	> 3.000 (+)	0.413 (+)	negative
2001. 3.12	0.186 (–)	> 3.000 (+)	0.327 (–)	negative
2003. 4. 8	0.142 (–)	0.827 (+)	0.072 (–)	negative

The specificity of the anti-HEV IgG and anti-HEV IgA assays was verified by absorption by the same recombinant ORF2 protein that was sued as the antigen probe or a mock protein obtained from the pupae of silkworm infected with non-recombinant baculovirus. The details were described in the text.

(IgG 1,289 mg/100 ml, IgA 191 mg/100 ml, IgM 212 mg/100 ml) and negative ANA (< 20).

Recently we evaluated the possibility of HEV infection in this case. Enzyme-linked immunosorbent assays (ELISAs) and RT-PCR were performed with the serial stocked serum samples as previously described (Okamoto et al. 2001; Mizuo et al. 2002). The specificity of the anti-HEV IgG and anti-HEV IgA assays was verified by absorption by the same recombinant ORF2 protein that was used as the antigen probe or a mock protein obtained from the pupae of silkworm infected with non-recombinant baculovirus. Briefly, when the OD value of the tested sample decreased to less than 30% of the original value after absorption with the recombinant ORF2 protein and remained greater than 70% of the original value after absorption with a mock protein, the sample was considered to be positive for anti-HEV. Although we could not detect HEV-RNA from them, IgA and IgG antibodies to HEV were detectable from the serum obtained at admission. The titer of IgA and IgG type anti-HEV antibodies decreased with time course (Table 3). Taken together, we have concluded that her possible diagnosis is acute hepatitis E.

Discussion

In industrialized countries, although anti-HEV has been detected in 4 to 36% of healthy individuals (Tam et al. 1991; Hsieh et al. 1999; Pina et al. 2000; Meng et al. 2002), sporadic cases of hepatitis E which were not associated with traveling to endemic area have rarely been reported (Schlauder et al. 1998; Erker et al. 1999; Hsieh et al. 1999; Schlauder et al. 1999; Zanetti et al. 1999; Li et al. 2000; Wu et al. 2000; Takahashi et al. 2002a).

Since HEV infection was not considered endemic in Japan, we did not examine it at the admission of this case. All the markers of hepatitis A, B, and C including other viral markers, such as Epstein-Barr virus, cytomegalovirus, and Varicella zoster virus were negative. Serum examination of autoantibodies revealed that ANA and ASMA were positive, and the titers of serum IgG and IgM levels were elevated. Other possible causes for hepatic failure (e.g., alcohol, drug-induced liver injury, etc.) were effectively ruled out.

From these data, we first diagnosed her clinically as an autoimmune hepatitis of acute onset form. When we looked over her clinical course, her initial response to PSL was not satisfactory, although subsequent corticosteroid pulse therapy was successful. We could successfully taper PSL without an exacerbation and finally discontinued it at 20 months after the administration. In classical AIH, the discontinuation of PSL is very difficult. The patient did not demonstrate any sign of a relapse after the cessation of PSL. In addition, her class II human leukocyte antigen (HLA) was DR6 and DR9, which is different from known major types in Japan (DR4 and DR8). Neither macroscopic nor microscopic pathological findings were compatible with classical AIH, but this might be due to the timing of performing liver biopsy (i.e., recovery phase of sever acute inflammation). Moreover, to the best of our knowledge, there are no established relationship between specific HLA haplotype and disease susceptibility of HEV infection as observed in chronic hepatitis C (Kondo et al. 2003).

In the recent study conducted in Japan, 11 (12.6%) of 87 patients who had previously been diagnosed as sporadic acute hepatitis of non-ABC etiology were found to be infected with HEV. The seroprevalence of antibodies against HEV in healthy individuals was reported to range from 1.9 to 14.1% in Japan (Li et al. 2000; Mizuo et al. 2002).

A genotype III strain of HEV has been isolated from a Japanese patient with acute hepatitis who had never been abroad. Also, several sporadic hepatitis E case has been reported (Takahashi et al. 2001; Suzuki et al. 2002; Takahashi et al. 2002b; Ohnishi et al. 2003; Yazaki et al. 2003).

These findings indicate that HEV infection may be endemic in Japan. We have considered possible involvement of HEV infection in the group of patients who had been diagnosed as acute hepatitis of unknown etiology in our hospital. Thus we examined the stocked sera obtained at the time of admission from these patients for HEV serological markers. We tested IgA, IgM

and IgG class antibodies to HEV by ELISAs and HEV-RNA by RT-PCR. Concerning the present case, we could not detect HEV-RNA in the any of stocked serum, although IgA, and IgG type antibodies to HEV were detected and their titers reduced with the clinical course. Therefore, we concluded that her diagnosis was acute hepatitis E. We assumed that positivity of ANA, ASMA and elevation of serum IgG level was due to the nonspecific reaction with viral infection or possibly specific reaction with some type of HEV infection. We could also raise the possibility that the acute infection with HEV could induce polyclonal immunoglobulinemia as observed in the current case, although the mechanism of this phenomenon is unclear.

In general, hepatitis type E is diagnosed by serological tests: i) detection of IgM type anti-HEV, ii) elevated titer of IgG type anti-HEV, or iii) positive viral RNA by RT-PCR. Since HEV is known to poorly tolerate the freeze and thaw (Bradley 1990), it cannot be detected if the serum is stocked under inappropriate condition. As a consequence, the prevalence of HEV infection could be understimated. IgA type anti-HEV antibody can be utilized as an additional confirmatory antibody for recent HEV infection (Chau et al. 1993; Tokita et al. 2003), and this case demonstrated positive reaction for it in an examination of stocked sera at admission. Also, although judged as negative, the titer of IgM type anti-HEV was weakly reactive on admission and was decreased after time course (Table 3).

HEV infection causes severe hepatitis during pregnancy with a mortality rate up to 58%. HEV infection during pregnancy has been reported to take a severe course in developing countries (Scharschmidt 1995; Purcell and Emerson 2001; Khuroo and Kamili 2003). In addition, zoonotic transmission as well as food-borne transmission of HEV is reported in Japan (Suzuki et al. 2002; Tei et al. 2003; Yazaki et al. 2003). Furthermore, the risk factors for acute HEV infection includes male with higher age, and residence in Northern part of Japan (Okamoto et al. 2003). Although this patient lived in Northern part of Japan, she had no known other risk factors. The infectious route and the cause of her severe clinical course are still unknown. Further studies regarding the role of HEV genotype and other possible factors are feasible to understand the pathogenesis.

In conclusion, we must take acute hepatitis type E into consideration for the diagnosis of cryptogenic acute hepatitis, including ones previously diagnosed as AIH of acute onset type like this case. Further studies are feasible to understand the clinical feature of HEV infection, especially the association with autoimmune hepatitis.

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