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Case report

A case of acute liver failure with echovirus infection diagnosed by a multi-virus real-time PCR system

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ABSTRACT

Background: Multi-virus real-time polymerase chain reaction (PCR) system is able to simultaneously detect 163 viruses using a multiplex Taqman real-time PCR system. We present a case of acute liver failure (ALF) of unknown etiology diagnosed with echovirus 30 infection via multi-virus real-time PCR.
Case presentation: A previously healthy 66-year-old man had a persistent fever and developed ALF of unclear etiology. Although viral infection was suspected, serological screening showed no evidence of acute viral infections such as hepatitis A, B, C and E, Epstein-Barr virus, herpes simplex virus, and varicella zoster virus. Multi-virus real-time PCR revealed the presence of enterovirus and echovirus 30 genomes, and reverse transcription-PCR using enterovirus-specific primers confirmed the presence of enterovirus genome in serum samples at the time of admission. Anti-echovirus antibody titers showed an increase in paired sera. In spite of multimodality treatment, the patient died due to multiple organ failure. Histological analysis in autopsy revealed extensive coagulative necrosis of the hepatocytes and immunohistochemical analysis showed the expression of enterovirus antigens in necrotic hepatocytes.
Conclusions: We present here a case of echovirus 30 associated with ALF. Multi-virus real-time PCR is useful for detection of virus for patients with ALF of unknown etiology suspected of harboring a viral infection.

Introduction

Acute liver failure (ALF) is a severe condition in which liver function rapidly deteriorates in individuals without prior history of liver disease. Mortality among ALF patients is high, often resulting from multiorgan failure and brainstem decompression due to cerebral edema [1,2]. While most cases result from hepatitis virus infection, autoimmune hepatitis, or drug-induced liver injury, no clear cause can be identified in approximately a third of patients in Japan [3]. Because no specific treatment is available for ALF patients with unknown etiology, such patients have poor prognosis [3]. Viral infection can lead to acute hepatitis and is associated with occasional ALF. Hepatitis viruses, such as hepatitis A, B, C, and E are the most common and important culprits, while several members of the Herpesviridae, such as herpes simplex, varicella zoster, Epstein-Barr and cytomegalovirus are also associated with ALF [2,3]. In addition, although rare, SEN virus and echovirus infections have been reported to cause ALF [4,5].

A multi-virus real-time polymerase chain reaction (PCR) system has been established to detect viruses in pathological specimens from patients with uncertain diagnosis [6]. This system is able to simultaneously detect more than 163 viruses (47 DNA viruses and

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116 RNA viruses) using a multiplex Taqman real-time PCR system. In this case report, we present a case with ALF who was diagnosed as having echovirus 30, a type of enterovirus, by multi-virus real-time PCR.

Case

A previously healthy 66-year-old man initially presented to his local hospital with fever and malaise. Since his blood test showed severe hepatic and renal dysfunction, he was referred to our hospital. On admission to our hospital, the patient's mental status was Glasgow coma scale 15 (eye opening: 4; verbal response: 5; best motor response: 6), hepatic encephalopathy grade 0, and his body temperature was 38.0 °C. A laboratory analysis revealed severe liver (total bilirubin,10.7 mg/dL; aspartate aminotransferase [AST], 11390 IU/L; alanine aminotransferase [ALT], 4682 IU/L) and renal dysfunction (blood urea nitrogen, 40.5 mg/dL; creatinine 6.29 mg/dL), coagulopathy (prothrombin [PT] activity, 10 %), thrombocytopenia ($4.4 \times 10^4/\mu L$) and inflammation (white blood cells, 10110/μL; C-reactive protein, 4.07 mg/dL) (Table 1). Although an infectious disease was suspected, serological viral markers such as hepatitis A, B, C, and E, herpes simplex virus, varicella zoster virus, and Epstein-Barr virus, human immunodeficiency virus and human T-cell leukemia virus type 1 were negative. He had no visible tick bite marks and no evidence of infection with severe fever with thrombocytopenia syndrome virus (SFTS). His only medical history was diabetes and hypertension, and review of the patient's medication history did not reveal a potential toxin. An abdominal and simple chest computed tomography scan revealed diffuse low absorption liver area considered fatty liver, mild obvious hepatic atrophy and a small amount of ascites and pleural effusion (Fig. 1). The patient was diagnosed with ALF based on criteria published by the Ministry of Health, Labour, and Welfare of Japan [3], although the etiology was unknown.

The clinical course of the patient is shown in Fig. 2. The patient received plasma exchange (PE) twice and hemodialysis filtration (HDF) five times for liver and renal failure beginning on the second day in the hospital. In addition, he was treated for disseminated intravascular coagulation for coagulopathy. Although AST and ALT levels decreased promptly, his level of consciousness gradually worsened from the third day of hospitalization. He was diagnosed with hepatic encephalopathy (hepatic encephalopathy grade 3) with high ammonia levels, but his level of consciousness had gradually improved following medication and HDF. We suspected that his ALF was caused by a viral infection due to the persistence of fever and white blood cell elevation. However, serological viral markers retested at day 21 revealed no evidence of any virus infection. On day 36 after admission, multi-virus real-time PCR was performed using stored serum samples from Days 1, 2, and 36. In the analysis, pan-enterovirus and echovirus 30 were positive in serum samples on Day 1 (1981 and 454 copy/μL, respectively) and Day 2 (294 and 149 copy/μL, respectively). Reverse transcription (RT)-PCR using enterovirus specific primer [7] and direct sequencing analysis confirmed the presence of echovirus 30 genome in serum samples obtained on Days 1 and 2. Neither multi-virus real-time PCR nor RT-PCR detected viral sequences in the Day

Table 1
Laboratory data at the time of admission.

Hematologic test			
		IgG (mg/dL)	1238 (861–1747)
White blood cells (/μL)	10110 (3300–8600)	IgM (mg/dL)	58 (33–183)
Neutrophils (%)	85	IgA (mg/dL)	154 (93–393)
Lymphocytes (%)	12	Anti-nuclear antibodies	<×80 (-)
Monocytes (%)	3	IgM-hepatitis A virus antibodies	<0.4
Eosinophils (%)	0	Hepatitis B surface antigen (IU/mL)	0
Basophils (%)	0	Hepatitis B core antibodies (COI)	4.4
Red blood cells ($\times 10^4/\mu L$)	471 (435–555)	IgM-hepatitis B core antibodies	(-)
Hemoglobin (g/dL)	15.7 (13.7–16.8)	Hepatitis C virus antibodies (COI)	0.1
Platelet count ($\times 10^4/\mu L$)	4.4 (15.8–35.8)	IgA-hepatitis E virus antibodies	(-)
Coagulation		Epstein-Barr virus	
Prothrombin activity (%)	10 (70–130)	Anti-VCA IgG	640
Activated partial thromboplastin time (s)	64.9 (26.9–38.1)	Anti-VCA IgM	<10
Fibrinogen (mg/dL)	85.6 (200–400)	Anti-EBNA antibodies	20
Antithrombin III (%)	29 (79–121)	Cytomegalovirus	
Chemistry		IgG	75
Total bilirubin (mg/dL)	10.7 (0.4–1.5)	IgM	(-)
Direct bilirubin (mg/dL)	7.1 (0.1–0.3)	Herpes simplex virus	
Aspartate aminotransferase (IU/L)	11390 (13–30)	IgG	≥128 (+)
Alanine aminotransferase (IU/L)	4682 (10–42)	IgM	0.16 (-)
Alkaline phosphatase (IU/L)	436 (106–322)	Varicella Herpes Zoster virus	
Lactate dehydrogenase (IU/L)	10064 (124–222)	IgG	39.5 (+)
γ-Glutamyltranspeptidase (IU/L)	165 (13–64)	IgM	0.26 (-)
Blood urea nitrogen (mg/dL)	40.5 (8–20)	Anti-human immunodeficiency virus-1 and 2 antibodies	0.07 (-)
Creatinine (mg/dL)	6.29 (0.65–1.07)		
C-reactive protein (mg/dL)	4.07 (0–0.14)	Human T-cell leukemia virus type 1	0.2 (-)
Procalcitonin (ng/mL)	1.06 (0–0.49)		
Total protein (g/dL)	5.9 (6.6–8.1)	Severe fever with thrombocytopenia syndrome virus	(-)
Albumin (g/dL)	3.2 (4.1–5.1)		
Sodium (mmol/L)	131 (138–145)		
Chloride (mmol/L)	94 (101–108)		
Potassium (mmol/L)	4.8 (3.6–4.8)		
Ferritin (ng/mL)	127900 (35.1–353.1)		
Ammonia (μmol/L)	75 (11–32)		
Hemoglobin A1c (%)	6.9 (4.9–6.0)		
Glucose (mg/dL)	53 (73–109)		

Normal ranges are shown in parentheses.

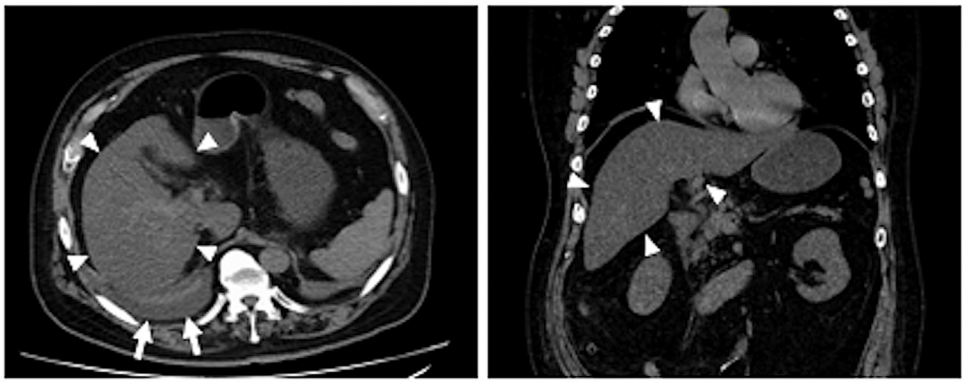

Fig. 1. Simple computed tomography scan. Axial and coronal slices are shown. Arrowheads indicate atrophic liver and arrows indicate ascites and pleural effusion.

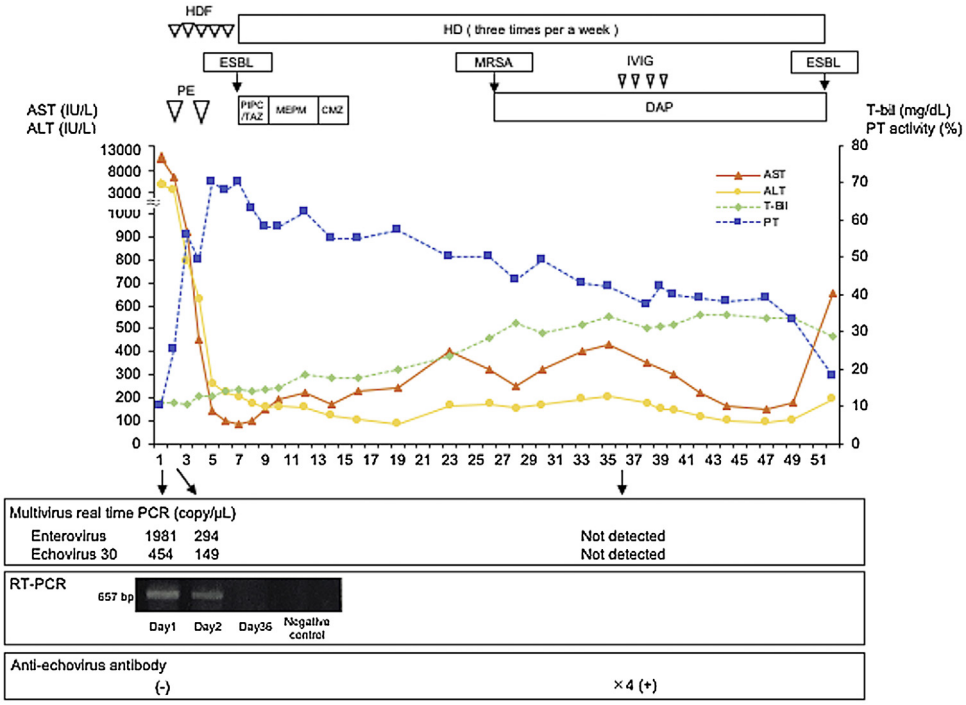

Fig. 2. Clinical course of the patient. HDF, hemodiafiltration; HD, hemodialysis; PE, plasma exchange; ESBL, extended-spectrum β-lactamase-producing *Escherichia coli*; MRSA, methicillin-resistant *Staphylococcus aureus*; PIPC/TAZ, piperacillin/tazobactam; MEPM, meropenem; CMZ, cefmetazole; DAP, daptomycin; IVIG, intravenous immunoglobulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; PT, prothrombin. Serum viral loads of enterovirus and echovirus 30 quantified by multi-virus real-time PCR, a cropping gel by reverse transcription (RT)-PCR of adenovirus gene (657 bp) and anti-echovirus antibody titers at the indicated time are shown.

36 serum samples. Anti-echovirus antibody titers showed an increase in paired sera at Days 1 and 36 (from negative to ×4). Because of the possibility of ALF induced by echovirus infection, immunoglobulin was administered for four days. He was also treated with antibiotics for repeated occurrence of catheter-induced sepsis such as MRSA infection. In spite of multimodality therapy, his liver failure did not improve, and he died 52 days after being admitted to our hospital due to multiple organ failure. An autopsy was carried out, and histopathological analysis revealed extensive coagulative necrosis of the hepatocytes from the center of the lobule to the periportal vein, consistent with ALF (Fig. 3A and B). Immunohistochemistry using anti-pan-enterovirus group B antibody [8] revealed that virus antigen was detected in necrotic hepatocytes (Fig. 3C).

Discussion

Multi-virus real-time PCR is able to simultaneously detect more than 163 viruses (47 DNA viruses and 116 RNA viruses) using a multiplex Taqman real-time PCR system [6]. The analysis was performed at National Institute of Infectious Diseases, Tokyo, Japan. Serum and multi-organ samples such as lung, heart, liver, brain, kidney and lymph node can be analyzed using the method. The list of the 163 viruses is shown in a previous report [6]. Using this system, herpes simplex virus 1, human herpesvirus 6 and parechovirus 3 were identified as causes of disease in patients with encephalitis [6]. The system also detected low titer of parvovirus B19, Epstein-Barr virus, and TT virus in liver tissues obtained from hepatitis patients [6]. In the present case ALF of unknown etiology,

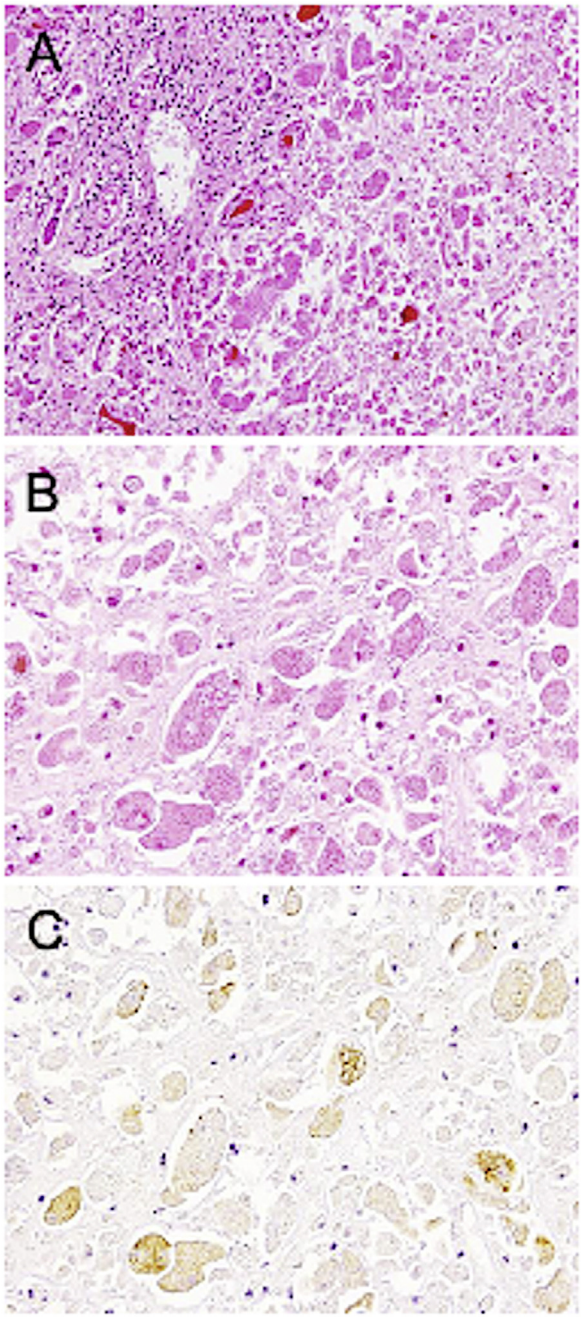

Fig. 3. Pathological findings of the liver in autopsy. In hematoxylin-eosin staining, massive necrosis of hepatocytes is observed (A and B). Immunohistochemistry showed positive signals in necrotic hepatocytes (C). Original magnifications are ×100 (A) and ×200 (B and C). (B) and (C) are serial sections.

echovirus 30 were detected by multi-virus real-time PCR system from the serum samples. No cross-reactivity with other enteroviruses such as polioviruses, coxsackieviruses, and other subtypes of echovirus was observed. Presence of enterovirus was also confirmed by conventional RT-PCR, and anti-echovirus antibody titers showed an increase in paired sera. Moreover, subsequently performed immunohistochemistry in autopsy revealed the expression of virus antigen in hepatocytes.

The relationship between enterovirus infection and ALF in the present case is unclear. Enteroviruses have a single-stranded, positive-sense RNA genome and are transmitted through the fecal-oral route [9]. Enterovirus is one of the most common viruses of meningitis in young infants and is also known to occasionally cause fulminant hepatitis in newborns [10]. Verboon-Maciolek et al. reported three young infants who developed liver failure caused by echovirus 20 infection [11]. Kawashima et al. reported that enterovirus was detected in serum and liver tissues by RT-PCR in nine out of 16 pediatric patients who showed abnormal liver function with unknown etiology [12]. In contrast to infants, enterovirus infections are often subclinical and rarely causes severe disease in adults. However, enterovirus infections had been reported to caused severe hepatitis in immunocompromised adults [5,13]. Martina et al. reported an adult case with ALF caused by echovirus 18 infection [5]. This patient had a history of non-Hodgkin lymphoma and was a hematopoietic stem cell transplant recipient, likely resulting in a severely compromised ability to mount a neutralizing antibody response to echovirus [5]. In regard to the present case, although the patient had a history of mild diabetes and obesity, he was never diagnosed with immunodeficiency, and no factors were found to pose a risk for infectious diseases.

Although we could not diagnose the cause of ALF in the present case, and the patient unfortunately died, the multi-virus real-time PCR system detected enterovirus and echovirus 30 infection, and subsequent immunohistochemical staining with anti-enterovirus antibodies in liver tissue showed positive findings, suggesting the possibility that echovirus 30 infection was associated with ALF. The system appears to be useful for detection of viruses in patients with ALF of unknown etiology, especially among those with suspected viral infection.

Author statement

All authors approved the final draft of the manuscript

Ethics approval and consent to participate

The study was performed in accordance with the World Medical Association Declaration of Helsinki and with approval of the local ethics committee.

Consent for publication

Written informed consent was obtained from the patient's next of kin for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Author contribution

YY, KM, KY, YA, YK. SU, HF, TN, EM, MY, TK, MT and AH analyzed and interpreted the patient data.HA and KC supervised the finding of this work. TW and HK performed the multi-virus real-time PCR and the histological examination of the liver. MI and CNH were major contributors in writing the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

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