A child with acute encephalopathy associated with quadruple viral infection

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INTRODUCTION

Acute encephalopathy (AE) is a neurological dysfunction that presents with altered consciousness, sometimes associated with neurological injury or death in young children (1, 2). Many etiologic factors have been identified, including infectious agents and genetic factors. AE in young children is often associated with virus infection. The risk factors for developing infantile AE upon virus infection remain to be determined. Here, we report an infant with AE co-infected with human herpesvirus-6 (HHV-6) and three picornaviruses, including coxsackievirus A6 (CVA6), Enterovirus D68 (EV-D68), and human parechovirus (HPeV). EV-D68 was vertically transmitted to the infant from his mother. CVA6 and HPeV were likely transmitted to the infant at the nursery school. HHV-6 might be re-activated in the patient. It remained undetermined, which pathogen played the central role in the AE pathogenesis. However, active, simultaneous infection of four viruses should have evoked the cytokine storm, leading to the pathogenesis of AE. Conclusion: an infant case with active quadruple infection of potentially AE-causing viruses was seldom reported partly because systematic nucleic acid-based laboratory tests on picornaviruses were not common. We propose that simultaneous viral infection may serve as a risk factor for the development of AE.

Keywords: acute encephalopathy, coxsackievirus A6, enterovirus D68, human parechovirus, human herpesvirus-6, risk factor, viral co-infection

BACKGROUND

PATIENT HISTORY

A previously healthy male infant aged 1 year and 9 months presented to a primary physician with fever (38.4°C). During a physical examination, the child had a convulsive seizure accompanied by flaccid paralysis of the limbs, upward tonic eye deviation, and tachycardia (190 beats/min). The subject was admitted to an emergency hospital. On arrival, the subject presented with high fever (39.3°C) and status epilepticus (Figure 1).

LABORATORY EXAMINATION, IMAGING, AND ELECTROENCEPHALOGRAPHY

Laboratory examinations revealed a white blood cell count of 19,060 cells/ml (neutrophils, 51.1%; lymphocytes, 43.4%; and monocytes, 4.0%); red blood cell count of 4.42 million cells/ml; white blood cell count of 19,060 cells/ml (neutrophils, 51.1%; lymphocytes, 43.4%; and monocytes, 4.0%); red blood cell count of 4.42 million cells/ml; and blood hemoglobin level of 9.5 g/dl. Most of the serum markers remained within the normal range, including C-reactive protein (CRP), total bilirubin (TB), creatinine phosphokinase (CPK), blood urate nitrogen (BUN), and creatinin. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were slightly elevated. Blood sugar was 240 mg/dl. Serum pro-inflammatory markers interleukin-6 (IL-6) and interleukin-1 beta (IL-1b) were 299 pg/ml (normal range, <4.0 pg/ml) and 1.4 pg/ml (normal range, <10 pg/ml), respectively. Cerebrospinal fluid (CSF) was colorless and transparent. CSF glucose, blood cell count, and total protein were within normal ranges. CSF IL-6 was 40 pg/ml, and IL-1β was undetectable. A cranial computed tomography scan and magnetic resonance imaging revealed no abnormalities. The electroencephalogram (EEG) was normal except for the presence of rhythmic delta waves. The patient was treated with anticonvulsant therapy, and fever was controlled with acetaminophen. After 5 days, the patient was transferred to an intensive care unit for further evaluation.

Pediatric acute encephalopathy (AE) was sometimes attributed to virus infection. However, viral infection does not always result in AE. The risk factors for developing infantile AE upon virus infection remain to be determined. Here, we report an infant with AE co-infected with human herpesvirus-6 (HHV-6) and three picornaviruses, including coxsackievirus A6 (CVA6), Enterovirus D68 (EV-D68), and human parechovirus (HPeV). EV-D68 was vertically transmitted to the infant from his mother. CVA6 and HPeV were likely transmitted to the infant at the nursery school. HHV-6 might be re-activated in the patient. It remained undetermined, which pathogen played the central role in the AE pathogenesis. However, active, simultaneous infection of four viruses should have evoked the cytokine storm, leading to the pathogenesis of AE. Conclusion: an infant case with active quadruple infection of potentially AE-causing viruses was seldom reported partly because systematic nucleic acid-based laboratory tests on picornaviruses were not common. We propose that simultaneous viral infection may serve as a risk factor for the development of AE.

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resonance imaging of the brain without contrast showed no particular findings. An electroencephalogram demonstrated diffuse, high-amplitude slow wave activity.

EXAMINATION OF INFECTIOUS AGENTS
The blood HHV-6 DNA load was $3 \times 10^2$ copies/ml, but HHV-6 was not detected in CSF. The plasma titer for anti-HHV-6 IgG was 1:60, and IgM was undetectable.

Enterovirus species were further examined because of the family history (detailed below) and a hand–foot–mouth disease (HFMD) outbreak in the nursery school where the subject was attending. In addition, regional surveillance revealed HFMD and herpangina outbreaks due to coxsackievirus A6 (CVA6) and enterovirus 71 (EV71) infections. Nucleic acid-based examination and virus isolation tests in tissue culture and suckling mice were performed (11). Enterovirus D68 (EV-D68) was detected in throat swab and stool specimens. HPeV was also positive for RNA extracted from the throat swab (12). Additionally, CVA6 was positive in the stool. A tissue culture-based virus isolation test detected HPeV-1 from the stool specimen (13). In the suckling mice-based virus isolation test, CVA6 was detected in stool specimen (14). However, CVA6, EV71, and EV-D68 were not detected by nucleic acid-based examination in CSF.

Beside these viruses, immunochromatographic tests were negative for rotavirus, HAdV, and RSV on admission. Nucleic acid-based tests were negative for HHV-7, Japanese encephalitis virus (JEV), vesicular stomatitis virus (VSV), and herpes simplex virus type (HSV) in blood and CSF. Bacterial cultures from blood and CSF were negative.

In summary, four viruses were detected: HHV-6 and three picornavirus species, namely, EV68, HPeV-1, and CVA6 (Figure 1; Table 1).

FAMILY HISTORY
Four days prior to onset of illness in the infant, his mother presented with cold symptoms. A day before and after the onset of illness in the infant, his father and older sister, aged 3 years and 4 months, also presented cold symptoms. PCR-based laboratory tests revealed that stool specimens from the mother were positive for EV-D68. Three enterovirus species were detected from his older sister: the stool specimen was positive for echovirus 18 (Echo18) and the throat swab was positive for both HPeV and rhinovirus (RhV). No virus was detected from the patient’s father (Figure 1; Table 1).

DISCUSSION
DIAGNOSIS, TREATMENT, AND OUTCOME
The differential diagnosis included acute encephalitis, acute meningitis, and non-infectious causes of AE such as toxic or metabolic encephalopathy. We diagnosed this patient as AE
associated with active infection of four viruses because of the low inflammatory markers. The seizure lasted 52 min, and was stopped by intramuscular administration of midazolam (0.22 mg/kg). The subject was then administered mannitol infusion 1 g/kg, methylprednisolone 30 mg/kg/day, and edaravone 1 mg/kg. Therapeutic normothermia (36.0°C) for 12.5 h was also performed. One day post-admission, the subject regained consciousness. However, drowsiness remained until 7 days post-admission. No apparent sequelae were recognized at discharge (Figure 1).

**Table 1 | Summary of viruses detected in the patient, his family, and regional surveillance.**

| Subject          | Detected virus | Specimen                  |
|------------------|----------------|---------------------------|
| Patient          | CAV A6         | Stool                     |
|                  | EV-D68         | Throat swab, stool        |
|                  | HHV-6          | Blood                     |
|                  | HPeV           | Throat swab, stool        |
| Mother           | EV-D68         | Stool                     |
| Father           | Not detected   | –                         |
| Older sister     | Echo18         | Stool                     |
|                  | HPeV           | Throat swab, stool        |
|                  | RhV            | Throat swab               |
| Regional surveillance | CVA6       | HFMD and herpangina patients |
|                  | EV71           | HFMD and herpangina patients |

CAV, coxsackievirus; Echo18, echovirus 18; EV71, enterovirus 71; EV-D68, enterovirus D68; HFMD, hand–foot–mouth disease; HHV-6, human herpesvirus-6; HPeV, human parechovirus; RhV, rhinovirus.

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**CONCLUDING REMARKS**

Picornavirus is often overlooked as an etiological factor for AE. We successfully detected three picornaviruses because we carefully monitored the regional surveillance data and used established, systematized laboratory examination protocols. Laboratory technology to detect pathogens at the nucleic acid level is advancing, and this may provide clues as to the etiology of AE.

**AUTHOR CONTRIBUTIONS**

KN performed the laboratory examinations, drafted, and reviewed the manuscript. MK, MM, SS, CO, and SH examined the subject of interest at the medical institution, performed medical practices, and collected clinical information and specimens. TK and JK coordinated the study. JK supervised data collection, drafted, reviewed, and finalized the manuscript. All authors approved the final manuscript as submitted.

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