Case Report

Severe acute encephalopathy related to human parainfluenza virus type 2 infection in an infant: a case report

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Introduction: We present here a rare case of severe acute encephalopathy with extra-pulmonary symptoms in a two-year-old girl caused by human parainfluenza virus type 2 (HPIV2) infection.

Case Presentation: The patient was brought in by ambulance, presenting with fever, hypoxia and generalized tonic-clonic seizure, and was admitted into Fukuyama Medical Center. She had a depressed level of consciousness with drowsiness. Her Glasgow coma score was 6. Based on the results of laboratory examinations, brain computed tomography, brain magnetic resonance imaging and electroencephalography, we diagnosed her with acute encephalopathy. Treatment was initiated with high-dose intravenous immunoglobulin, methylprednisolone pulse therapy and edaravone along with mechanical ventilation. We confirmed HPIV2 infection using samples of sputum from the intra-tracheal tube, throat swab and blood using next-generation sequencing and the PCR method. After continued steroid and anti-inflammatory therapy, the patient recovered completely.

Conclusion: Extra-pulmonary symptoms in parainfluenza viral infections are rare. HPIV2 infection can cause severe acute encephalopathy via a systemic immunological reaction along with airway symptoms.

Keywords: acute encephalopathy; edaravone; human parainfluenza virus type 2 infection/seizure; intravenous immunoglobulin; intravenous steroids.

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Abbreviations: AST, aspartate aminotransferase; CMV, cytomegalovirus; CSF, cerebrospinal fluid; CT, computed tomography; EBV, Epstein–Barr virus; EEG, electroencephalography; HHV, human herpesvirus; HN, haemagglutinin-neuraminidase; HPIV2, human parainfluenza virus type 2; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; NGS, next-generation sequencing; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

The GenBank/EMBL/DDBJ accession number of the haemagglutinin-neuraminidase coding region sequence obtained in this study is LC050829.

Introduction

Human parainfluenza viruses (HPIVs) are respiratory viruses that commonly cause respiratory infections in children (Henrickson, 2003). In recent years, the importance of extra-pulmonary symptoms of HPIV infection has become evident, and severe cases of HPIV-related encephalitis have been reported (Kim & You, 2012; Abenheim Halpern *et al.*, 2013).

Here, we report a case of a two-year-old girl who was diagnosed with HPIV-induced acute encephalitis, presenting with fever, seizure and reduced level of consciousness. HPIV2 was detected using next-generation sequencing (NGS), and other viruses that typically cause encephalopathy, such as the Epstein–Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), human herpesvirus (HHV)6 and HHV7, were not detected.

Case report

A two-year-old girl was brought to our hospital by ambulance with fever, hypoxia and generalized tonic-clonic seizure. She had a mild nasal discharge and cough for half a day before admission. She had a history of severe neonatal asphyxia and an Apgar score of 2/8 and had been delivered at the gestational age of 32 weeks. However, routine follow-up revealed good development, with normal findings on brain magnetic resonance imaging (MRI) and electroencephalography (EEG).

At the time of hospital admission, the patient also had laryngomalacia, for which tracheotomy was performed. She had a depressed level of consciousness with drowsiness. Her Glasgow coma score was 6. The seizure resolved with anticonvulsant treatment, but the consciousness level did not improve. Essential laboratory data are shown in Table 1. Serum levels of aspartate aminotransferase (AST; 99 IU l⁻¹), lactate dehydrogenase (LDH; 466 IU l⁻¹),

ferritin (143.3 ng ml⁻¹) and amyloid A (153.3 µg ml⁻¹) were elevated; therefore, we considered that she had severe systemic inflammation. The cerebrospinal fluid (CSF) was sampled on the day of admission, and the cell count was 1 cell µl⁻¹. Rapid diagnostic tests using nasal or throat swabs for respiratory syncytial virus (RSV), influenza virus, adenovirus, and human metapneumovirus were all negative. Immediately after hospitalization, although brain MRI did not suggest inflammation (Fig. 1a), brain computed tomography (CT) showed brain oedema. Based on these findings, as well as considering the persisting depressed level of consciousness, we diagnosed severe acute encephalopathy. Prior to starting treatment, we obtained the guardian's informed consent for obtaining a nasal swab, intra-tracheal sputum sample and blood samples on the day of admission. Therapy was initiated with 0.5 g D-mannitol kg⁻¹ per dose four times per day for reducing cerebral oedema and 0.12 mg midazolam kg⁻¹ h⁻¹ continuously for preventing seizure. Further, 1 g high-dose intravenous immunoglobulin (IVIG) kg⁻¹ day⁻¹, 30 mg methylprednisolone pulse therapy kg⁻¹ day⁻¹ for 3 days, 500 U thrombomodulin alfa day⁻¹, and 0.5 g edaravone kg⁻¹ dose⁻¹ twice a day were concomitantly administered, similar to the therapy for influenza-virus-associated encephalopathy. Mechanical ventilation was started for the respiratory symptoms. Further, we administered 150 mg cefotaxime kg⁻¹ day⁻¹ since the white blood cell count and C-reactive protein levels were elevated. However, bacterial cultures for blood and CSF were negative, and throat swab culture detected normal flora.

After the initiation of treatment, the patient's consciousness improved briefly; however, on day 4 of admission, her consciousness worsened and the laboratory data suggested severe systemic inflammation. MRI on day 6 showed high-signal areas in the anterior lobes; therefore, we restarted methylprednisolone pulse therapy and increased the D-mannitol dose (Fig. 1b). Subsequently,

Table 1. Clinical laboratory data from the acute and convalescent phases

Item	Acute phase (day 1, admission)	Acute phase (day 2)	Convalescent phase (day 28)
White blood cell (mm ⁻³)	16 400	14 900	8000
Neutrophils (%)	19.0	67.0	72.0
Platelet count (mm ⁻³)	21.1 × 10 ⁴	18.0 × 10 ⁴	33.9 × 10 ⁴
Fibrin degradation products (µg dl ⁻¹)	5.5	7.1	1.4
D-Dimer (µg ml ⁻¹)	2.1	2.4	0.2
Total protein (g dl ⁻¹)	7.2	7.9	7.9
Albumin (g dl ⁻¹)	4.3	3.4	3.9
Aspartate aminotransferase (IU l ⁻¹)	99	81	73
Alanine aminotransferase (IU l ⁻¹)	35	36	27
Lactate dehydrogenase (IU l ⁻¹)	466	422	496
C-reactive protein (mg dl ⁻¹)	4.8	30.4	0.9
Ferritin (ng ml ⁻¹)	143.3	234.8	28.5
Serum amyloid A (µg ml ⁻¹)	153.3	1384.8	67.1

Fig. 1. Diffusion-weighted images of brain MRI. (a) High-signal regions in the anterior lobes on day 1. (b) Increase in the spread of high-signal areas, especially in the left cerebral hemisphere on day 6.

her consciousness and laboratory data improved gradually (Table 1). The results of EEG and brain MRI showed considerable improvements before the patient's discharge on day 42 of hospital admission. Follow-up examination at 3 months after the initial admission revealed good physical and mental development.

Investigations

We performed a comprehensive examination of pathogen genomes using NGS with MiSeq (Illumina) using the samples of nasal swab, intra-tracheal sputum and blood taken on the day of admission. Briefly, an RNA-seq library was prepared from extracted total RNA using a ScriptSeq

Fig. 2. Phylogenetic tree based on the HN coding region (305 nt) using Molecular Evolutionary Genetics Analysis (MEGA) software version 5 (Tamura *et al.*, 2011). Evolutionary distances were estimated using Kimura's two-parameter model, and a phylogenetic tree was reconstructed using the neighbour-joining method (Kimura, 1980; Saitou & Nei, 1987). The reliability of the tree was estimated using 1000 bootstrap replications (shown as percentages at nodes). Scale bar indicates nucleotide substitutions per site.

v2 RNA-Seq Library Preparation kit (Illumina), followed by 150-mer paired-end *de novo* sequencing with a MiSeq Reagent kit v2 (Illumina); the raw short reads (approx. 1 million reads per sample) were analysed using the MePIC server to characterize potential pathogens, as previously described (Takeuchi *et al.*, 2014). Sequence analysis by NGS suggested that this sample contained HPIV2. Therefore, we confirmed the presence of HPIV2 in the nasal swab using the conventional PCR method with specific primers (Bellau-Pujol *et al.*, 2005). Next, we performed phylogenetic analysis based on the haemagglutinin-neuraminidase (HN) coding region of HPIV2 (305 bp) using Molecular Evolutionary Genetics Analysis (MEGA) software version 5 (Tamura *et al.*, 2011) (Fig. 2). HN glycoprotein is a major antigen in HPIV. In addition, HN glycoprotein might function as a major antigen. In phylogenetic analysis, the reference sequences of the HN coding region were obtained by BLAST. Levels of the anti-HPIV2 antibody elevated 40-fold in the haemagglutination inhibition test after IVIG and methylprednisolone pulse therapy were started; however, they were not detected at the three-month follow-up. No pathogenic viruses, such as EBV, CMV, VZV, HHV6, HHV7 or HSV, were isolated or detected from the patient's serum (McIver *et al.*, 2005).

Diagnosis

Acute encephalopathy related to human parainfluenza virus type 2 infection.

Treatment

D-Mannitol at a dose of 0.5 g kg^{-1} four times per day, $0.12 \text{ mg midazolam kg}^{-1} \text{ h}^{-1}$ continuously, $1 \text{ g high-dose IVIG kg}^{-1} \text{ day}^{-1}$, $30 \text{ mg methylprednisolone pulse therapy kg}^{-1} \text{ day}^{-1}$ for 3 days, $500 \text{ U thrombomodulin alfa day}^{-1}$ and $0.5 \text{ mg edaravone kg}^{-1} \text{ dose}^{-1}$ twice a day. They were concomitantly administered, similar to the therapy for influenza-virus-associated encephalopathy.

Outcome and follow-up

Full recovery without sequelae.

Discussion

We have reported here the case of a two-year-old girl diagnosed with acute severe encephalopathy related to HPIV2 infection.

Recently, several extra-pulmonary symptoms caused by respiratory viruses have been reported (Eisenhut, 2006; Kim & You, 2012; Miyamoto *et al.*, 2013; Abenheim Halpern *et al.*, 2013). RSV infections affecting other organs have been reported (Miyamoto *et al.*, 2013), with the RSV antigen detected in the CSF, myocardium, liver and peripheral blood. Further, a case of acute encephalopathy associated with human metapneumovirus infection in a Japanese three-year-old girl was reported in 2014, in which the human metapneumovirus was detected using PCR from a throat swab but not from CSF, with elevated

levels of inflammatory cytokines in the CSF and blood samples (Niizuma *et al.*, 2014).

HPIVs are negative-strand RNA viruses from the *Paramyxoviridae* family (Henrickson, 2003) with four types; HPIV2 is a member of the genus *Rubulavirus* (Henrickson, 2003). These HPIVs typically cause respiratory symptoms ranging from mild upper respiratory tract infections to severe lower respiratory tract illness, including croup syndromes, bronchiolitis, and pneumonia. HPIV2 is an important causative agent of upper and lower respiratory tract infections and febrile illness in children as well as severe lower respiratory tract infection in all age groups (Henrickson, 2003).

Recently, several cases of acute encephalopathy related to HPIV have been reported, especially in Asian countries (Kim & You, 2012; Abenheim Halpern *et al.*, 2013). In these reports, HPIV was detected from patients' nasal swabs. In these cases, anti-inflammatory treatment using prednisolone was effective, similar to that in our case. In our case, the HPIV2 gene was detected in the patient's sputum samples, nasal swabs and blood samples using NGS. However, her anti-HPIV2 antibody levels were not elevated 3 months after admission. The anti-HPIV2 antibody level was 40-fold higher after the immunoglobulin product was used. We considered that the negative antibody was possibly due to the immunosuppressive effect of high-dose corticosteroids and high-dose IVIG.

In Japan, paediatric cases of acute encephalopathy, especially influenza-virus-associated encephalopathy, are very severe and often fatal (Kawada *et al.*, 2003). It is well known that in cases of influenza-virus-associated encephalopathy, viral RNA is rarely detected in the CSF; moreover, viral antigens are not detected in the brain on autopsy (Kawada *et al.*, 2003). Cytokine storm as a systemic immune response is considered to be a cause of influenza-virus-associated encephalopathy, wherein elevated levels of inflammatory cytokines, such as IL-1, IL-6 and TNF, are considered to cause the breakdown of the blood-brain barrier, leading to brain oedema (Kawada *et al.*, 2003). In our patient, the elevated levels of AST, LDH and ferritin suggested a systemic immune response; further, the increased serum amyloid A level strongly suggested an inflammatory cytokine response involving IL-1, IL-6 and TNF (Sztejn *et al.*, 1982; Patel *et al.*, 1998).

In conclusion, we have presented a case of severe acute encephalopathy associated with HPIV2. We detected the HPIV2 gene using NGS performed using the patient's sputum samples, nasal swabs and blood samples; however, CSF samples tested negative for the virus. Acute encephalopathy was diagnosed based on the patient's symptoms, laboratory data, and findings of brain CT, MRI and EEG. Anti-inflammatory and steroid treatment was effective, and the patient recovered without any sequelae. Our experience suggests that HPIV2 can indirectly cause extra-pulmonary illnesses via systemic inflammation, similar to that in influenza-virus-associated encephalopathy.

Ethical statement

The antigen investigation was approved by the Ethics Committee of the National Hospital Organization Fukuyama Medical Center. The patient's parents provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association.

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