Detection of Group A Rotavirus RNA and Antigens in Serum and Cerebrospinal Fluid from Two Children with Clinically Mild Encephalopathy with a Reversible Splenial Lesion

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SUMMARY: We report on two children with mild encephalopathy with a reversible splenial lesion associated with group A rotavirus (GARV) infection. We examined stool, serum, and cerebrospinal fluid samples to determine the presence of the GARV VP7 gene and GARV antigen by reverse-transcription PCR and enzyme-linked immunosorbent assay, respectively. GARV antigen was detected in stool samples from both patients. The GARV G genotype was G9 in one child and G3 in the other. GARV antigens were also found in both serum samples. However, the GARV VP7 gene was detected in only one serum sample, which was collected on the first day of symptomatic illness. Neither GARV antigen nor the VP7 gene was detected in cerebrospinal fluid samples. Both patients had excellent outcomes. Our results suggest that the reversible splenial changes in our patients might have been caused by indirect effects to the central nervous system subsequent to viral infection.

INTRODUCTION

Rotavirus is a major causative agent of gastroenteritis, which occurs mainly in children. Seven species of rotavirus, A, B, C, D, E, F, and G, have been described. Most cases of rotavirus gastroenteritis in children are caused by group A rotavirus (GARV). There is increasing concern about acute encephalopathy, which is a complication of rotavirus infection (1,2). In 2004, Tada et al. identified mild encephalopathy/encephalitis with a reversible splenial lesion (MERS), which is characterized by transient splenial lesions with high-signal intensity in diffusion-weighted magnetic resonance imaging (MRI), a mild clinical course, and a good outcome as a new type of acute encephalopathy (3). Although there are some reports of MERS associated with rotavirus infection (4–9), only a few studies identified rotavirus antigens or genes in patients with MERS (6).

Two patients with rotavirus encephalopathy presented with reversible splenial lesions of high-signal intensity in diffusion-weighted MRI. We investigated the presence of viral antigens and genes in samples of their stool, serum, and cerebrospinal fluid (CSF) using techniques that were previously used for patients with rotavirus gastroenteritis with or without neurological symptoms (10,11). Rotavirus RNA was not detected in the CSF, although the antigen was detected in the sera of both children. This suggests that the reversible splenial changes seen in our patients were caused by indirect effects to the central nervous system subsequent to viral infection.

PATIENTS AND LABORATORY INFORMATION

Case 1: A previously healthy 3-year-old boy experienced recurrent vomiting and watery diarrhea for 2 days. He was admitted to a nearby hospital for dehydration. He developed a disturbance of consciousness soon after a drip infusion was started, and he was transferred to our institute on the 3rd day of illness. At admission, he was agitated and could not make eye contact. He had a temperature of 36.7°C, a heart rate of 116 beats/min, a blood pressure of 112/70 mmHg, and a respiratory rate of 24 breaths/min. Blood tests revealed hyponatremia with 128 mEq/L, compensated metabolic acidemia (pH, 7.419; pCO2, 26.6 mmHg; HCO3, 16.8 mmHg; and base excess, −5.9 mmHg). His blood sugar and ammonia levels were normal. CSF analysis did not show pleocytosis, protein elevation, or a reduction of sugar. GARV antigen was detected in a stool sample with a commercial kit (Dipstick Eiken Rota; Eiken Chemical Co., Tokyo, Japan). Acute encephalopathy associated with rotavirus infection was diagnosed. MRI of the brain showed an intensified signal in the splenium of the corpus callosum (Fig. 1A). The boy was treated with intravenous osmotic diuretic fluid containing isotonic sodium. His mental status improved on the 2nd day of admission. A second brain MRI performed on the 9th day of illness showed no abnormal signals. The intensified signal seen on diffusion-weighted MRI had disappeared (Fig. 1B). He was discharged with no neurological se-
**Case 2:** A 3-year-old boy was admitted to a nearby hospital because of pyrexia and recurrent vomiting for 2 days. He had been receiving speech training for a speech delay beginning 1 year prior to admission. Soon after admission, he no longer responded to his mother’s voice. He was transferred to our institute on the 3rd day of illness. At admission, he was crying, and eye contact was lost. He had a temperature of 38.0°C, a heart rate of 140 beats/min, a blood pressure of 88/50 mmHg, and a respiratory rate of 20 breaths/min. Blood tests revealed hyponatremia with 129 mEq/L, compensated metabolic acidemia (pH, 7.476; pCO$_2$, 23.2 mmHg; HCO$_3$, 16.7 mmHg; and base excess, −4.7 mmHg). His blood sugar and ammonia levels were normal. CSF analysis did not show pleocytosis, protein elevation, or a reduction of sugar. GARV antigen was detected in his stool. Diffusion-weighted MRI conducted on the day of admission showed an intensified signal in the splenium of the corpus callosum (Fig. 1C). Osmotic diuretic and isotonic fluid infusions were started once he was diagnosed with rotavirus encephalopathy. His mental status improved the day after he was admitted. A brain MRI performed on the 10th day of illness showed no abnormal signals (Fig. 1D). He was discharged with no neurodevelopmental regression.

**RESULTS**

*Detection of GARV RNA and antigen:* We investigated the presence of GARV VP7 genes by reverse-transcription PCR and GARV VP7 antigens by enzyme-linked immunosorbent assay in seven samples (two CSF samples, three serum samples, and two stool samples), as previously described (10,11). The results are shown in Table 1. The genotype of GARV in the stool of Case 1 was identified as G9. The serum sample from Case 1 was also positive for GARV antigen, but the GARV VP7 gene was not detected. The GARV detected in the stool of Case 2 was identified as G3 genotype. In Case 2, GARV antigens were detected in the sera on the 3rd and 4th days of illness. In the serum sample taken on the 3rd day of illness, reverse transcription PCR of the VP7 gene revealed that the virus was genotype G3, the same as in the stool sample. In both cases, neither the antigen nor the gene was detected in CSF samples.

**DISCUSSION**

GARV is a common cause of severe gastroenteritis in children, and 4% of affected children develop central nervous system symptoms (1). There have been many reports of rotavirus-associated encephalopathy, which are on a clinically broad spectrum, ranging from benign convulsions to fatal outcomes (1). The question of whether these neurological complications are a direct or indirect effect of viral infection of the central nervous system has been discussed. The hypothesis that these changes are a direct effect of viral infection is supported by several studies that detected rotavirus RNA in the CSF (1,2,10,11). We previously examined the CSF from six patients with seizures accompanied by rotavirus infection and detected viral genes in all of the samples (10). Moreover, Lynch et al. reported the presence of rotavirus RNA in the CSF of a 2.5-year-old girl whose only symptom was consciousness disturbance (1). However, it is difficult to associate neurological symptoms with the existence of rotavirus RNA in the CSF.

The fact that rotavirus antigen was detected both in the stool and sera in this study suggests the spread of rotavirus from the gut to the bloodstream. The GARV RNA was detected only in one serum sample of Case 2 that had been collected early in the acute phase. If the serum of Case 1 had been collected earlier, the GARV RNA might have been positive (12). Neither case

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**Table 1. Detection of GARV VP7 gene (in stool, serum, and CSF samples) and antigen (in serum and CSF samples)**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Stool</th>
<th></th>
<th>Serum</th>
<th></th>
<th>CSF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day of collection</td>
<td>VP7 (G type)</td>
<td>Day of collection</td>
<td>VP7 (G type)</td>
<td>ELISA (OD)</td>
<td>Day of collection</td>
</tr>
<tr>
<td>Case 1</td>
<td>4</td>
<td>G9</td>
<td>6</td>
<td>–</td>
<td>0.373</td>
<td>3</td>
</tr>
<tr>
<td>Case 2</td>
<td>3</td>
<td>G3</td>
<td>3</td>
<td>G3</td>
<td>0.492</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>NE</td>
<td></td>
<td></td>
<td>0.391</td>
<td></td>
</tr>
</tbody>
</table>

Day of collection was counted from the onset of diarrhea or/and vomiting.
Cut-off for positive ELISA value is an OD of 0.3.
NE, not examined; –, negative.
cases to date, the outcome was excellent unless cerebelli-
made. In all of the rotavirus infection-associated MERS
in enough cases to allow any firm conclusions to be
presence of GARV in the CSF has not been determined
by direct invasion, but by indirect effects. However, the
MERS associated with rotavirus infection is caused not
GARV RNA in the CSF in these cases suggests that
consciousness, the same as in our cases. The absence of
than ours, and GARV RNA was not detected (6). The
MERS (16). CSF was examined in only one case other
ble to rule out hyponatremia as a contributing factor of
vomiting and diarrhea, preceded MERS, and GARV an-
ted in stool samples. It is notable that
neurological symptoms. A combination of virological investigations, radiological
studies of cytokines and free radicals will
showed spread or replication of rotavirus in CSF.
Transient high signals in the splenium of the corpus
callosum on diffusion-weighted MRI have been report-
ed in adult patients, such as those taking antiepileptic
drugs or suffering from hypoglycemia (13–15). Recent-
ly, this MRI finding was observed in children with en-
cephalopathy or encephalitis who had a mild clinical
course and good outcome. MERS has become one of
the entities of acute encephalitis/encephalopathy in
pediatrics (3). Although a variety of viruses, such as
those causing influenza, mumps, and varicella, can
cause this condition, the pathophysiologic mechanisms
remain unknown. Several reports describing MERS
related to rotavirus infection are listed in Table 2. In all
of these cases, symptoms of gastroenteritis, such as
vomiting and diarrhea, preceded MERS, and GARV anti-
gens were detected in stool samples. It is notable that
no. 5–7 in Table 2 were reported as acute cerebellitis.
These three cases presented first with seizures and/or
consciousness disturbances like the other six cases, but
cerebellar symptoms developed later. Mutism was the
most common and interesting symptom among these
cases. We noted hyponatremia in both of our cases.
Takanashi et al. reported that the serum sodium levels
of patients with MERS were significantly lower in those
with upper respiratory infection, and that it is not possi-
bile to rule out hyponatremia as a contributing factor of
MERS (16). CSF was examined in only one case other
than ours, and GARV RNA was not detected (6). The
only symptom that patient had was mild disturbance of
consciousness, the same as in our cases. The absence of
GARV RNA in the CSF in these cases suggests that
MERS associated with rotavirus infection is caused not
by direct invasion, but by indirect effects. However, the
presence of GARV in the CSF has not been determined
in enough cases to allow any firm conclusions to be
made. In all of the rotavirus infection-associated MERS
cases to date, the outcome was excellent unless cerebelli-
tis developed.
In a previous report, increased levels of serum
cytokines were observed in Reye syndrome patients with
GARV infection (17). Moreover, increased levels of free
radicals in serum and CSF samples were reported in
patients with GARV infection with seizures (18). These
observations support the hypothesis of an indirect effect
of rotavirus infection on neurological symptoms. A
combination of virological investigations, radiological
analyses, and studies of cytokines and free radicals will
provide clues as to the pathophysiology of MERS with
GARV infection.

**Conflict of interest** None to declare.

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splenial lesions in children with “benign convulsions with gas-
Neuro, 40, 131–133.
rotavirus-associated encephalopathy with reversible restricted
diffusion in splenium of the corpus callosum: importance of
diffusion weighted imaging and apparent diffusion coefficient

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**Table 2. Previously published reports of MERS associated with rotavirus infection**

<table>
<thead>
<tr>
<th>Study (year and reference no.)</th>
<th>Patient age, sex</th>
<th>Symptoms of CNS</th>
<th>Serum sodium (mEq/l)</th>
<th>Laboratory detection of rotavirus</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Kobata, et al. 2002 (4)</td>
<td>2 y, F</td>
<td>seizure, consciousness disturbance</td>
<td>136</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>2. Natsume, et al. 2007 (5)</td>
<td>2 y, F</td>
<td>clumping seizure</td>
<td>not shown</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>3. Fukuda, et al. 2009 (6)</td>
<td>2 y, M</td>
<td>consciousness disturbance</td>
<td>130</td>
<td>Antigen (+)/G3</td>
<td>NE</td>
</tr>
<tr>
<td>4. Suzuki, et al. 2009 (7)</td>
<td>1 y, F</td>
<td>seizure, consciousness disturbance</td>
<td>137</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>5. Takanashi, et al. 2010 (8)</td>
<td>3 y, F</td>
<td>consciousness disturbance, mutism, ataxia, dysarthria</td>
<td>not shown</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>6. Takanashi, et al. 2010 (8)</td>
<td>4 y, F</td>
<td>seizure, consciousness disturbance, mutism, tremor, ataxia</td>
<td>not shown</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>7. Kubota, et al. 2011 (9)</td>
<td>1 y, F</td>
<td>seizure, consciousness disturbance</td>
<td>normal</td>
<td>Antigen (+)/NE</td>
<td>NE</td>
</tr>
<tr>
<td>8. This report 2011 (9)</td>
<td>3 y, M</td>
<td>consciousness disturbance</td>
<td>128</td>
<td>Antigen (+)/G9, P[8]</td>
<td>Antigen (+)/RNA (−)</td>
</tr>
<tr>
<td>9. This report 2011 (9)</td>
<td>3 y, M</td>
<td>consciousness disturbance</td>
<td>129</td>
<td>Antigen (+)/G3, P[8]</td>
<td>Antigen (+)/G3</td>
</tr>
</tbody>
</table>

NE, not examined; M, male; F, female; CNS, central nervous system.