Enterovirus infections are associated with white matter damage in neonates

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Aim: To explore the imaging findings of neonatal infants infected with enteroviruses.

Methods: A retrospective study was conducted on 12 patients who were diagnosed with encephalitis caused by enterovirus. Clinical presentation, cranial ultrasonography (cUS), magnetic resonance imaging (MRI) findings and neurodevelopment outcome of 12 cases were analysed.

Results: Twelve infants, with a gestational age of 35 to 39 weeks, presented at 36 to 41 weeks postmenstrual age with clinical symptoms of enterovirus infections. Ten of 12 neonatal infants had a fever and 4 of 12 presented with a sepsis-like illness. cUS in one preterm infant showed periventricular echogenicity. Neonatal MRI confirmed white matter changes in 12 infants. Follow-up of infants were 18 months. Outcome was variable with cerebral palsy in 2 infants and normal neurodevelopment outcome in 10 infants.

Conclusions: Enterovirus may cause severe central nervous system infection in the neonatal period. The neuroimaging studies are informative and should be a part of care for infants with enteroviruses.

Key words: enterovirus infection; neonate; white matter damage.

Enteroviruses are known to cause neonatal infections and most likely to occur during the summer and fall months. Enteroviruses are spread mainly from person to person by the fecal–oral or oral–oral routes. Clinical manifestations may greatly vary from non-specific febrile illness to severe life-threatening disease. Neonates with enterovirus infections are at high risk of developing a sepsis-like condition that may progress to encephalitis, hepatitis and coagulopathy. Many neonates are hospitalised because of fever, irritability, poor suckling and lethargy. Neonatal enterovirus encephalitis leads to severe white matter damage and adverse neurological sequelae.

White matter damage, which includes focal abnormalities, thinning of the corpus callosum, and diffuse, excessive, high-signal intensity, is emerging as the leading central nervous system (CNS) lesion detected by magnetic resonance imaging (MRI) among infants born prematurely. Studies demonstrated an association between this injury and adverse motor and cognitive neurodevelopmental outcomes. Previous studies identified perinatal clinical risk factors, including prematurity, hypoxia, ischaemia and fetal-maternal infection.

During the assessment of early neonatal period by MRI, white matter damage is significantly detected as focal, non-cystic, hyperintense areas on T1-weighted MRI scans, whereas volume loss and signal changes typically are later findings seen among near-term infants. In studies of correlations of MRI findings and pathologic findings for premature newborns, focal hyperintense lesions on T1-weighted MRI scans most closely corresponded to areas of gliosis.

However, until now, there have been few reports that describe the imaging findings of enterovirus infections in neonatal period. The aim of the study was to describe the clinical presentation, cranial ultrasonography (cUS) data, MRI findings and neurological outcome of 12 neonates with non-polio enterovirus encephalitis.

Methods

Between May 2011 and August 2011, 12 infants with enterovirus infections were admitted to the neonatal ward of the...
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Children’s Hospital, Zhejiang University School of Medicine. The diagnosis of enterovirus infection was proven by a positive enterovirus polymerase chain reaction (PCR) on faeces or cerebrospinal fluid (CSF).

**cUS**

cUS was performed using a Philips scanner (Philips Medical Systems, Best, The Netherlands) with a transducer of 7 MHz crystals.

**MRI**

MRI was performed using a 1.5 Siemens Magnetom magnetic resonance system (Siemens AG, Erlangen, Germany) located in the radiology unit. The magnet had a 2.54-m-long and 0.68-m-wide bore. Initial MRI included sagittal T1-weighted (repetition time (TR)/time of echo (TE) 500/8.4 ms, 210 × 210 mm field of view (FOV)) and T2-weighted spin echo (TR/TE 4000/89 ms, 210 × 210 mm FOV) sequences and diffusion-weighted imaging (DWI) (TR/TE 2900/92 ms, 210 × 210 mm FOV, and b value = 800 s/mm²). Follow-up MRI included the following sequences: sagittal T1-weighted and T2-weighted spin echo sequences and fluid-attenuated inversion recovery (TR/TE 7500/129 ms, 230 × 180 mm FOV). Slice thickness varied with 5 mm for axial and 3 mm for sagittal images.

**Viral diagnosis**

The viral diagnosis was confirmed by a positive enterovirus reverse transcription (RT)-PCR on stool or CSF. Molecular typing was accomplished from stool. This RT-PCR test was performed with all prototypes of enterovirus. Viral RNA was extracted from 100-μL infected tissue culture fluid using TRIzol reagent (Sangon, Shanghai, China). Five microlitres of eluted RNA was used for amplification using an enterovirus real-time RT-PCR kit (Zhijiang Biotech, Shanghai, China). Cycling conditions for RT-PCR amplification were 10 min at 45°C for RT, and one cycle for 15 min at 95°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C.

**Neurological and developmental follow-up**

Neurodevelopment outcome was assessed at 6 and 18 months after birth. A full neurological assessment and the general movements were performed. Cerebral palsy (CP) was diagnosed after 2 years of age by neurologic examination in the neonatal follow-up or rehabilitation clinics. CP was defined as appearance in early life of a persistent but unchanging disorder in tone, movement and posture that was attributable to a non-progressive disorder of the brain.

**Results**

**Clinical data**

Six preterm (at 35 to 36 weeks) and six full-term infants, of whom six were born by vaginal delivery, presented with clinical symptoms of a viral infection during the summer. All bacterial cultures remained negative. The infected infants were predominantly male with a male to female ratio of 2:1. Eleven of neonates developed enteroviral disease in the first week of life and one full-term infant became ill within the first 2 weeks of life. Ten of 12 infants had fever. The common symptoms like poor suckling, irritability, diarrhoea or rash were discovered in six infants. Three preterm and one full-term infants presented with a sepsis-like illness leading to hepatitis and a coagulation disorder with thrombocytopenia in the first week after birth (Table 1). The platelet count ranged from 12 to 34 × 10⁹/L in infants with a sepsis-like illness. CSF analysis was performed in all infants. Eight patients showed predominantly elevated leukocyte count (40–660 × 10⁶/L) and increased monocytes in initial CSF sample. Protein and glucose levels remained in the normal range.

**Result of cUS**

cUS was performed during the first week after birth and was initially normal in 11 patients but one preterm infant. cUS showed extensive periventricular echogenicity.

**Result of MRI**

MRI was performed between 3 and 25 days after onset of clinical symptoms. Appearances of diffuse high-signal intensity of white matter on T1-weighted sequences with local areas of low-signal intensity were detected in nine infants. Hypointensity on T2-weighted sequences were seen in four infants. DWI, performed in 10 infants, showed abnormal high-signal intensity restricted diffusion in the periventricular white matter, splenium of corpus callosum, posterior limb of internal capsule and deep white matter of hemisphere (Table 2). In the follow-up MRI examination of five patients, the lesions completely disappeared within 2 weeks to 2 months. In a series of patient 3 who underwent follow-up MRI at 2 months, it showed mild abnormality of the periventricular white matter.

**Virology**

Enterovirus was identified by PCR of the stool sample from all infants. In nine of these patients, eventovirus was also detected by PCR of CSF. Isolation of enterovirus was given from stool in eight infants. Three different serotypes of enteroviruses were represented. Coxsackie type B1 was isolated from two patients, and echovirus 30 and 31 were identified in three patients (Table 1). Interestingly, the two infants that developed CP were infected with echovirus 30.

**Neurodevelopmental outcome**

Neurodevelopment outcome was assessed at 6 and 18 months after birth. Two children experienced development of CP. One child with CP (patient 3, Fig. 1) had extensive changes in the white matter, specifically well visualised on DWI. Another child showed high-signal intensity in periventricular white matter on DWI (patient 7, Fig. 2). Ten infants displayed normal development at 18 months.

**Discussion**

Enterovirus infections are a significant cause of morbidity and mortality throughout the world. Enteroviruses are known to target the CNS and are responsible for numerous clinical
manifestations, including encephalitis and meningitis. The CNS disease in newborns caused by enteroviruses may also progress to meningoencephalitis with the appearance of seizures and focal neurological deficits.

In this study, the most common clinical features associated with enterovirus infection were fever, diarrhoea, irritability and frequent rashes. Neonates with enterovirus infection are at high risk of developing a sepsis-like illness because they are unable to mount an effective immune response. In a neonate with clinical signs more likely connected with enterovirus infection, lumbar puncture should be necessarily considered to do to obtain CSF. In the study, 12 neonates with enterovirus carried out lumbar puncture and resulted pleocytosis in 8 of 12 infants, and the protein and glucose levels were always normal.

Although cerebral infection has been frequently diagnosed in neonates with enterovirus infection, reports about CNS damage are limited. Recently reported imaging features of enterovirus 71 and echovirus 7 encephalitis in older children showed hyperintense changes in the pons and medulla on T1- and T2-weighted images. In our study, we found mild to severe white matter abnormalities in our patients with enterovirus encephalitis. Diffuse signal intensity changes of the white matter and punctate white matter lesions were seen on T1- and T2-weighted sequences. Abnormal high-signal intensity restricted diffusion in the periventricular white matter, splenium of corpus callosum, posterior limb of internal capsule and deep white matter of cerebral hemisphere could be seen on DWI. DWI and apparent diffusion coefficient measurements provided additional information compared with other sequences in diagnosing white matter damage during the acute phase. The neonates with extensive white matter abnormalities did not present with clinical seizures, suggesting that white matter abnormalities may remain undetected, depending on the threshold of MRI chosen. MRI should be performed in neonates suspected of a severe enterovirus infection. The severity of the imaging abnormalities correlated with later neurodevelopmental outcome. The two infants who developed CP had extensive change in the white matter, specifically well visualised on DWI. Ten patients showed normal development at ages 18 months.

There was only one report about white matter damage in neonatal enterovirus meningoencephalitis and neurodevelopment. It reported six neonates with enterovirus meningoencephalitis, including three preterm infants (28 to 33 weeks) and three term infants. MRI confirmed mild to severe white matter damage in all infants. Three infants had developed an adverse outcome. In our study, only two infants developed CP. The differences may be related to gestation ages of patients and the difference in vulnerability of the white matter during maturation. Our 12 infants were born at term or developed the infection at term age equivalent, whereas three infants with abnormal neurodevelopment were born at 28 to 33 weeks of the recent literature.

The pathogenesis of progressive white matter damage in the enterovirus infection is unknown. For some infants, the association between recurrent infection and white matter damage in the postnatal period may be related to direct invasion by microorganism. In infants without CNS infection,
however, the white matter damage may be attributable to inflammatory cytokines that are generated during periods of ischaemia. The hypothesis is supported by experimental animal models that suggest a relationship between cytokine responses and white matter damage and studies in premature human infants that showed a relationship between inflammatory cytokine levels and white matter damage.

Termination of the inflammatory process occurs by production of anti-inflammatory cytokines by activated T cells and/or monocytes after elimination of the virus. In the immature brain, cytokines and perivascularly accumulated lymphocytes may lead to white matter damage. Interestingly, the two infants that developed CP were the only two infected with echovirus 30. However, it is unclear whether the virulence of

Table 2  Imaging result of cUS and MRI outcome of neonates with EV infections

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>cUS</th>
<th>MRI</th>
<th>DWI-MRI</th>
<th>Outcome at 18 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>High SI in the periventricular WM on T1WI</td>
<td>Not available</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>High SI in the periventricular WM on T1WI</td>
<td>Punctate high SI in the left cerebral peduncle</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>High SI in left deep WM at the level of the centra semiovale on T1WI</td>
<td>High SI in left deep WM along the corticospinal tracts at the level of the centra semiovale</td>
<td>Cerebral palsy</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Excessive high SI in the periventricular WM on T1WI</td>
<td>High SI in the left cerebral peduncle</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Excessive high SI in the periventricular WM on T1WI</td>
<td>High SI in the cerebral ventricular</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>High SI in deep WM of hemisphere on T1WI and T2WI</td>
<td>High SI in splenium of corpus callosum</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>Punctate high SI in periventricular WM on T1WI</td>
<td>High SI in periventricular WM</td>
<td>Cerebral palsy</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>High SI in the periventricular WM on T1WI, hypointensity on T2WI</td>
<td>Not available</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>Cystic</td>
<td>High SI in the pericerebellum WM on T1WI</td>
<td>High SI in the pericerebellum</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>Normal</td>
<td>Punctate high SI in periventricular WM on T1WI</td>
<td>High SI in left posterior limb of internal capsule</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>Normal</td>
<td>Hyperintensity on T1WI and hypointensity on T2WI in deep WM</td>
<td>Punctate hypointensity in the cerebellum</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>Normal</td>
<td>High SI in periventricular WM on T1WI</td>
<td>High SI in the periventricular and the corpus callosum</td>
<td>Normal</td>
</tr>
</tbody>
</table>

cUS, cranial ultrasound; DWI, diffusion-weighted imaging; EV, enterovirus; MRI, magnetic resonance imaging; SI, signal intensity; T1WI, T1-weighted imaging; T2WI, T2-weighted imaging; WM, white matter.

Fig. 1  MRI of patient 3. Patient 3, born at postconceptional age of 38 weeks; MRI performed at age of 11 days. At the level of the centra semiovale, abnormal hyperintensity (white arrow) is present on the T1-weighted image (a), and abnormal hyperintensity (white arrow) is present in left deep white matter along the corticospinal tracts on diffusion-weighted imaging (b). MRI, magnetic resonance imaging.
the echovirus 30 was associated with more severe white matter damage.

The diagnosis of enterovirus infection is made on the basis of a positive enterovirus isolation or PCR in clinical samples. A laboratory diagnosis was obtained by PCR of the stool in 12 infants and from CSF in 9 cases, suggesting that stool PCR is a relatively sensitive method in the age group of diagnosis of enterovirus disease. Cerebral infection that is generally limited to the involvement of the cerebral parenchyma without the involvement of the meningitis can result in negative enterovirus PCR of the CSF. In 5 of 12 cases, isolation of enterovirus from stool was positive. According to the recent literature, PCR assay is a more sensitive method than virus isolation and leads to more rapid diagnosis of enterovirus disease. In the near future, it is certain that PCR detection of enterovirus in various fluids will play an increasingly important role in the diagnosis of enterovirus disease.

As found in our study, enterovirus can lead to severe encephalitis associated with matter damage in the neonatal period. White matter damage can be visualised with cUS, but the information obtained by DWI-MRI tends to be more detailed. Thus, the neuroimaging studies are informative and should be a part of care for infants with enteroviruses. However, it is still unclear from the data that our study has much prognostic value or that it impacts the management of the infants with enteroviruses, and we should do more findings to advance the field.

Acknowledgements

The work of the authors is supported by grants from National Natural Science Foundation of China (81170601) and sponsored by Zhejiang Provincial Program for the Cultivation of High-level Innovative Health talents.
References


Painting by Ibrahim Almaouie from Operation Art 2012.