Enterovirus 75 Encephalitis in Children, Southern India

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Recent outbreaks of enterovirus in Southeast Asia emphasize difficulties in diagnosis of this infection. To address this issue, we report 5 (4.7%) children infected with enterovirus 75 among 106 children with acute encephalitis syndrome during 2005–2007 in southern India. Throat swab specimens may be useful for diagnosis of enterovirus 75 infection.

Sequences of enterovirus 75 (EV75) were first identified in cerebrospinal fluid (CSF), stool samples, and throat samples obtained during 1974–2000 in Ethiopia, Oman, Bangladesh, and the United States; the new enterovirus serotype was proposed in 2004 (*1*). Manifestations include upper respiratory tract infections and acute flaccid paralysis. In 2005–2006, EV75 was associated with aseptic meningitis in Spain (*2*,*3*). Enteroviruses have long been associated with encephalitis, but recent outbreaks of EV71 in Southeast Asia have highlighted the diagnostic difficulties that may be encountered (*4*–*6*). We report EV75 associated with encephalitis in India.

The Study

We prospectively studied children ≤16 years of age who came to the Pediatric Department at the Vijayanagar Institute of Medical Sciences, Bellary, Karnataka, southern India, with acute encephalitis syndrome during October 2005–December 2007. Children with acute fever and symptoms with an onset <14 days before coming to the hospital, a neurologic illness, and ≥1 of the following signs (change in mental status including confusion, disorientation, coma, or inability to talk; new onset of seizures excluding simple febrile seizures; photophobia, headache, or meningitis) were recruited into the study. Children with laboratory-confirmed *Plasmodium falciparum* parasitemia or a history of neurologic conditions and those whose parents removed them from the hospital were excluded. The study protocol was reviewed and approved by the ethics committees of Vijayanagar Institute of Medical Sciences and the University of Liverpool, UK. Informed consent was obtained from parents or guardians.

For each child, a completed medical history was obtained and a detailed physical examination was performed. Samples were obtained for routine diagnostics. Rectal and throat samples were also collected after December 2005. Swabs were placed in sterile vials containing viral culture media (Dulbecco's modified Eagle's medium). All samples were frozen at –20°C, stored at –70°C, and transferred to diagnostic laboratories.

To detect enteroviruses, including EV75, in throat swab extracts, RNA was extracted by using the Chemagic Viral DNA/RNA kit (Chemagen AG, Baesweiler, Germany) and a Kingfisher mL Magnetic Extractor (Thermo Fisher Scientific Inc., Waltham, MA, USA). Pan-enterovirus reverse transcription–PCR (RT-PCR) was then performed by using primers specific for the 5′ untranslated region (5′-ATT GTC ACC ATA AGC AGC CA-3′ and 5′-CCT CCG GCC CCT GAA TGC GGC TAA T-3′); these primers produced a 154-bp product (*7*). Enteroviruses identified by pan-enterovirus RT-PCR were typed by nucleotide sequencing of the viral protein 1 (VP1) region (*8*,*9*).

To further type enteroviruses, phylogenetic analysis was performed on all nucleotide sequences (from this study and others obtained from GenBank) by using MEGA4 software (*10*) (www.megasoftware.net). The remaining swab transport medium was filtered, and 100 μL of filtrate was aliquoted onto each of 3 tissue culture plates containing rhabdomyosarcoma, Vero, and 293T cell lines for virus isolation. Cell lines were chosen to facilitate culturing of a range of viruses that may have been responsible for the clinical spectrum of illness seen.

Because CSF volume for virus isolation was limited to a 100-μL sample, this sample was placed on rhabdomyosarcoma cells for isolation and identification of enteroviruses. All CSF and serum samples were tested by using an immunoglobulin M capture ELISA to detect antibodies to Japanese encephalitis virus (JEV) and dengue virus, which circulate in the study region (*11*). If a sufficient amount of CSF remained, RNA was extracted by using the Viral RNA Mini Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions for pan-enterovirus RT-PCR, as described above and for a JEV RT-PCR (*7*,*12*). Plasma samples were also tested for chikungunya virus by using RT-PCR (*13*).

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