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An Outbreak of Echovirus Meningitis in Children

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An outbreak of aseptic meningitis in children as evidenced by increase in the number of admissions in a tertiary care hospital is described. Clinical data and stool samples were collected from 25 hospitalized infants and young children. The stool samples were subjected to virological investigations. Fever and vomiting were the commonest symptoms. Cerebrospinal fluid (CSF) showed lymphocytic pleocytosis in majority of cases. Of the 25 stool samples, 14 showed an enterovirus specific cytopathogenic effect (CPE) in rhabdomyosarcoma (RD) cell line. All the 14 samples were positive for enterovirus RNA by reverse transcription-polymerase chain reaction (RT-PCR). Partial sequencing of the Virion protein 1 (VPI) region of the enterovirus genome carried out on the first 7 isolates revealed 5 isolates to be echovirus serotype 4 and one each to be echovirus serotypes 3 and 30. All children showed a rapid recovery and were discharged within 3 days of admission.

Key words: Aseptic meningitis, Enterovirus, Outbreak.

Enteroviruses are the commonest cause of aseptic meningitis with majority of cases attributable to Coxsackie and Echo viruses(1-3). Enteroviral meningitis occurs sporadically or as epidemics(1), with some of the largest outbreaks caused by echoviruses(4).

Though outbreaks of enterovirus meningitis are commonly reported from industrialized countries there are few documented reports from India. We report

here an outbreak of echovirus meningitis in Vellore and surrounding areas as evidenced by a sudden increase in the number of infants and children admitted to the pediatric wards of our hospital.

Subjects and Methods

An outbreak of enterovirus meningitis was suspected when there was a sudden increase in number of patients admitted with a diagnosis

of aseptic meningitis in the pediatric wards of our hospital. Data were retrospectively analyzed for 25 such children (age 0-12 years) admitted from July through September 2002. Clinical and laboratory data were abstracted from the clinical records of the patients into a standardized proforma. Cerebrospinal fluid (CSF) examination for cytology, glucose and protein content, Gram stain and bacterial culture was done in all cases. In addition, stool samples were subjected to virological investigations.

Virus Culture

Stool samples were collected in a tube with viral transport medium (VTM) and transported to the laboratory on wet ice at +4°C immediately. Samples were processed by centrifugation at 2000 rpm for 10 minutes at +4°C and 0.2 ml of the supernatant obtained was inoculated (by enhanced adsorption method)(5) onto susceptible cell culture monolayers (Vero and rhabdomyosarcoma [RD]) and incubated at 37°C. The tubes were observed daily for cytopathogenic effect (CPE) characteristic of enterovirus, up to 7 days for RD and upto 10 days for Vero. Cell cultures showing enterovirus specific CPE were passed again into the cell line for confirmation. Supernatants of cell lines showing CPE upon the second passage were stored at -60°C for confirmation by PCR.

RT-PCR

RNA was extracted from the cell culture supernatants showing enterovirus-specific CPE using a commercial kit (QIAGEN QIAamp Viral RNA Mini kit). A random primed two-step nested RT-PCR was carried out as previously described(6). Amplified products were visualized by agarose gel electrophoresis with the expected product size being 304 base pairs.

Partial Sequencing of Virion Protein 1 (VP1) Region

From the RNA extracts, synthesis of cDNA and PCR amplification were carried out separately. Following cDNA synthesis(6) PCR amplification using 3 deoxyinosine containing degenerate primers (012, 040 and 011)(7) was performed to generate an approximately 450 base pair product (spanning the VPI-2A region of the enterovirus genome) that was detected by ethidium bromide stained agarose gel electrophoresis. Cycle sequencing of the amplified product and identification of the serotype was done as described(8).

Results

Twenty five children (18 males and 7 females) admitted to the hospital from the 29th of June 2002 to 4th of September 2002 were investigated. Agewise, 4 were infants, ten aged between 1 to 5 years, 9 aged between 6 to 10 years and 2 were older than 10 years of age. Most of the subjects were residents of Vellore and Tiruvannamalai districts in Tamilnadu or the adjoining Chittoor district in Andhra Pradesh.

The presenting signs and symptoms in the subjects are shown in *Table I*. Vomiting was the commonest symptom seen in 88% of the subjects followed by fever in 80%. Meningitis was suspected because of the presence of meningeal signs (neck stiffness or kernig sign), headache or persistent vomiting in older children and a bulging anterior fontanelle, irritability and incessant crying in children less than two years of age. All the patients were treated with intravenous antibiotics pending the results of the CSF culture. All children showed rapid improvement in symptomatology and 23 children were discharged from hospital within 3 days of admission and the remaining 2 within 5 days.

The CSF was clear in appearance in all

TABLE I—Presenting Signs and Symptoms in the Study Subjects (n =25).

Sign or symptom	No.(%)
Vomiting	22 (88)
Fever	20 (80)
Headache	16 (64)
Meningeal signs	14 (56)
Diarrhea	3 (12)
Seizures	2 (8)
Irritability	2 (8)
Other symptoms*	2 (8)

* Abdominal pain (1), blurring of vision(1).

children. Total leukocyte counts ranged from 20 to 760 per cu mm (median count 130 per cu mm). There was lymphocytic predominance in 20 subjects, whereas 5 had neutrophilic predominance. The glucose concentration was normal in all subjects and the protein concentration was less than 45 mg/dL in all but one patient, in whom it was 108 mg/dL. Gram stain of the CSF smears showed occasional pus cells with no bacteria. CSF cultures were negative for any bacterial growth.

Of the 25 stool samples collected, 14 samples showed enterovirus-specific cytopathogenic effect in only RD cell line, which were confirmed by a second passage. None of these showed any cytopathogenic effect in Vero cell line cultures. Random-primed two step RT-PCR carried out on the RD cell line supernatants showing enterovirus-specific CPE, detected the presence of the 304 base pair enterovirus-specific product in all 14 samples.

The first seven of the 14 isolates amplified using primers (011, 012 and 040) directed against the VPI-2A region generated an approximately 450 base pair product. All these isolates sequenced using 2 (011, 012) of the

above-mentioned 3 primers could be assigned to a particular serotype by sequencing. Of the 7 isolates, 5 were identified as echovirus serotype 4 and one each was identified as echovirus serotypes 3 and 30 respectively.

The pairwise identity score for the enterovirus isolates from this study with the respective homologous GenBank sequences ranged from 83 % to 89%.

Discussion

The etiological diagnosis of aseptic meningitis is seldom attempted in most sporadic cases due to cost constraints and lack of laboratory facilities. This outbreak provided us with the opportunity to conduct an investigation including virological studies. The similarity in clinical presentation and the high rates of detection of enteroviruses from our cases indicate that we did encounter an outbreak of meningitis due to echovirus. Data from the previous year also showed an increase in cases of aseptic meningitis during June and July. This suggests that there is a seasonal increase in cases of aseptic meningitis during these months in our area as also reported in North America and Europe. The majority of cases were due to echovirus type 4. The two cases with detection of echovirus 3 and 30 might either represent the sporadic cases or could be contributory to the outbreak as epidemics due to multiple serotypes of echovirus have been described previously(4). The study documents the occurrence of an outbreak of aseptic meningitis due to echovirus serotype 4. However, the study has limitations as it was hospital-based and virus typing was done in only small number of cases. Thus it does not document the extent of the outbreak or accurately describe all the serotypes involved and their proportional contribution to the outbreak.

Key Messages

- An outbreak of echoviral meningitis is described. The seasonality was similar to that reported from Europe and North America.
- Seasonality and dramatic improvement after performance of a lumbar puncture are additional criteria that may be used in differentiating viral from bacterial meningitis.
- Virological investigations on stool samples provide an etiological diagnosis.

Though excretion of enteroviruses may be prolonged in the stool of children with asymptomatic infection(9), isolation in a child with aseptic meningitis makes it very likely that the enterovirus is the etiological agent, especially when it is isolated from multiple cases in an outbreak situation. Stool samples are useful for the detection of nonpolio enteroviral infections(10-12) and sometimes serve as the only clue to diagnosis(1). Enterovirus specific cytopathogenic effect in cell cultures in a majority of samples, presence of an enterovirus specific PCR signal in all the culture positive stool samples and detection of potentially neurotropic enterovirus serotypes upon sequencing, especially echovirus serotypes 4 and 30, which are known to be associated with outbreaks, substantiates that the viruses obtained from stool samples did not represent asymptomatic excretion but the agents involved in the outbreak of aseptic meningitis.

The use of more than a single cell line for virus culture as in our study, increases the sensitivity of detection of enteroviruses(13). RD cell line is more sensitive for isolation of echoviruses(14) which can explain echovirus isolates growth only in RD in our study. Use of RD cell line also increases the speed of virus isolation(14). Sequencing using primers as done in our study is useful for the rapid determination of epidemiological relatedness of viruses isolated during an outbreak(7).

In conclusion, our study documented an

outbreak of echovirus serotype 4 in Vellore and surrounding areas and also suggested the benign self-limiting course of the illness.

Contributors: NS was responsible for all the virological investigations and drafted the manuscript; JXS contributed to clinical data and specimen collection and reviewed the manuscript; SRV contributed towards DNA sequencing of virus isolates and reviewed the paper; GS contributed to critical review of the manuscript and coordinated the concept and design of the study; TSV contributed to concept design and to reviewing of the manuscript; AR contributed to sample processing and culture work; TC initiated and coordinated the study and was responsible for manuscript preparation.

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