Review

Enteroviral encephalitis in children: clinical features, pathophysiology, and treatment advances

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Enteroviruses (EVs) have emerged as one of the important etiological agents as a causative organism for encephalitis, especially in children and adults. After the first report of EV encephalitis cases in 1950s, there have been increasing reports of regular outbreaks of EV encephalitis worldwide. Enteroviruses are RNA viruses of the family Picornaviridae that consists of more than 100 serotypes, which are characterized by a single positive-strand genomic RNA. The clinical features are pleomorphic and can be accompanied by mucocutaneous manifestations or isolated encephalitis only. The incidence of encephalitis in EV infection is reported to be about 3% and is associated with high mortality and morbidity. A number of newer therapeutic agents have been used in EV encephalitis with variable results. This review will focus on clinical features, pathophysiology, and newer treatment modality in EV encephalitis.

Keywords: Enteroviruses, Encephalitis

Introduction

Encephalitis is defined by the presence of an inflammatory process of the brain in association with clinical evidence of neurologic dysfunction.[1] A wide range of organisms have been implicated as a cause of encephalitis ranging from bacteria, viruses, *Rickettsiae*, spirochetes, fungi, and protozoa.[2] Out of these, viruses are the most prevalent cause all over the world. Previously, Herpes simplex virus (HSV1 and HSV2), varicella zoster virus, cytomegalovirus (CMV), Japanese encephalitis virus, dengue virus, chikungunya virus, West Nile virus, and Nipah virus were thought to be responsible for the major part of viral encephalitis cases and epidemics; but now enteroviruses (EVs) are emerging as one of the important etiological agent as a causative organism for encephalitis.[2–4]

Enteroviruses are RNA viruses of the family Picornaviridae that consists of more than 100 serotypes, which are characterized by a single positive-strand genomic RNA. The EV group includes 12 species, which include 4 species of human EVs A–D.[5] Viruses previously classified as EVs, namely echovirus (ECV) 22 and 23, recently have been reclassified as human parechoviruses (HPeVs), a different genus within the Picornaviridae family consisting of 16 different HPeV types.[6] Encephalitis is a rare presentation of EV infection, but EV serotypes such as coxsackievirus (CV) A9, A10, and B5; ECVs 4, 5, 9, 11, 19, and 30; and EV 71, 75, 76, and 89 have been reported in encephalitis cases and epidemics from various parts of the world.[7–12] In parechoviruses, HPeV3 has been shown to play an important role in severe neonatal central nervous system (CNS) infections.[6]

Epidemiology

Many viruses in EV group can cause encephalitis that varies by distribution and occurrence in different geographical regions.[13] Infection is seasonal in temperate climates (summer and autumn) but high round the year in tropical and subtropical countries. Various studies from India, Kuwait, and European countries report the prevalence of EV in encephalitis cases to be as high as 21–22% in encephalitis endemic areas.[7,14,15] Neonates and infants infected with CV have been shown to be extremely susceptible to myocarditis, meningitis, and encephalitis with a subsequent mortality rate as high as 10%.[16]

EV encephalitis and meningo-encephalitis cases were first reported in late 1950s (most cases in 1959) and they were of non-polio EV and ECV.[17,18] Since then many serotypes have been recognized as the causative agent for encephalitis.

In recent years, EV 71, which is an aggressive neurotrophic serotype of EV, has been recognized as

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a causative organism for a rapidly fatal rhombencephalitis in south-east Asian countries. Enterovirus 71 was first isolated and characterized from cases of neurological disease in California in 1969.[19] Besides causing encephalitis, EV 71has been recognized as a causative organism for epidemics of hand–foot–mouth disease in association with encephalitis.[20] A high mortality rate (19.3%) has been reported from Taiwan in children below 5 years of age.[21]

Besides EV 71, there are reports of other EVs as a cause of encephalitis from various parts of the world. In a prospective study conducted in south India, 4.7% patients with a clinical signs of acute encephalitis syndrome (AES) were positive for EV 75.[22] Another prospective study from northern India over a period of 2 years in children with acute encephalitis identified ECV 21 as the main causative agent of encephalitis in about 51.8% isolates followed by ECV 1, CV B1, EV 75, CV B5, and ECV 19.[9] Another study conducted in Spain found that 10.8% isolates from the patients with aseptic meningitis were positive for EV 75.[23]

Isolates from 306 patients with acute encephalitis during an outbreak of viral encephalitis in northern India identified EV in 21.6% of cases; Sequencing and phylogenetic analyses of PCR products from 89.3% specimens showed similarity with EV 89[24] and EV 76 sequences.[12]

A long-term surveillance study over a period of 11 years (2002–2012) in eastern China identified CVs A9, B1, B2, B3, B4, and B5 and ECVs 3, 4, 6, 9, 14, 25, and 30 from the unknown etiological encephalitis cases. Notably, CV B4 was identified for the first time in this region. Also, during 2002–2004 and 2010–2012, ECV 30 was found to be the periodically predominant serotype in the patients with enteroviral encephalitis.[24]

There are reports of association of echoviruses (ECHO-Vs) with aseptic meningitis. ECHO-Vs are highly infectious and preferentially target infants and young children.[16] Parechoviruses (HPeV), which were initially classified among the EV genus (ECVs 22 and 23), have been recently reclassified as parechoviruses based on their different biological properties.[25] These agents have been found to be associated with encephalitis and white matter injury in neonates and younger children with similarities to EV infections.[16]

Unfavorable outcomes (death or neurological sequelae) of enteroviral encephalitis have been associated with younger age (<4 years), high peak leukocyte counts (>13 000/mm^3), seizures, skin rash, myoclonic jerks, lower CSF viral yield rate, and EV 71 infection.[21]

Immuno-pathogenesis

The EV enters the human host through the GI or respiratory tract. The cell surfaces of the GI tract serve as viral receptors, and initial replication begins in the local lymphatic GI tissue. The virus seeds into the bloodstream, causing a minor viremia on the third day of infection. The virus then invades organ systems, causing a second viremic episode on days 3–7. This second viremic episode is consistent with the biphasic prodromal illness.[26]

The infection can progress to CNS involvement during the major viremic phase or at a later time while invading the motor neurons of the anterior horn cells of the spinal cord. It can progress to other CNS regions, including the motor cortex, cerebellum, thalamus, hypothalamus, midbrain, and medulla, causing death of neurons and paralysis. Neuropathy occurs due to direct cellular destruction. Antibody production in response to enteroviral infections occurs within the first 7–10 days in the lymphatic system of the GI tract, before invasion of the CNS tissue.[26]

Infants retain transplacental immunity for the first 4–6 months of life.[23] Most of the fatal cases of EV 71 occur in children aged <3 years.[26] The primary site of attack is the central nervous system, particularly the brainstem. They develop rapidly progressive sympathetic hyperactivity, pulmonary edema (PE) and/or pulmonary hemorrhage, and cardiopulmonary collapse. Overwhelming PE is the leading cause of death in these children.[26] The systemic inflammatory mediators increase in patients with PE appears to be triggered by persistent sympathetic activation as a consequence of direct brainstem destruction by the virus. Leukocytosis and thrombocytosis were significantly more frequent among patients with PE. A significant elevation of plasma interleukin (IL)-10, IL-13, and interferon (IFN)-g levels has been observed in patients with PE. Patients with PE also had lower circulating CD4+ T cells, CD8+ T cells, and natural killer (NK) cells.[26]

Clinical Features

Most EV infections are asymptomatic or result in only mild illnesses, such as non-specific febrile illness or mild upper respiratory tract infections. However, EVs can also cause a wide variety of clinical illnesses, which are mild to potentially life threatening in spectrum.[27,28] Coxsackievirus infection during pregnancy has been linked to an increase in spontaneous abortions, fetal myocarditis, and neurodevelopmental delays in the newborn.[16] Parechovirus infections are mostly enteric and often associated with mild gastrointestinal and respiratory symptoms, although severe neonatal diseases including sepsis, meningitis, encephalitis, and hepatitis have been described.[29]

CNS manifestations of enteroviral infection

Enterovirus can cause various diseases in the nervous system, including aseptic meningitis, acute paralysis,

encephalitis, meningo-encephalomyelitis, poliomyelitis-like paralytic disease, opsoclonus-myoclonus syndrome, benign intracranial hypertension, and brainstem encephalitis.[30] Also, a number of delayed neuropathologies have been associated with previous CV infection, including schizophrenia, encephalitis lethargica, and amyotrophic lateral sclerosis.[16]

Enteroviral encephalitis presents with wide range of symptoms, alone or a combination of them: fever, headache, lethargy, drowsiness, altered sensorium, coma, splenomegaly, hepatomegaly, acute onset of flaccid muscle weakness, hyporeflexia or brisk deep tendon reflexes, positive meningeal signs and signs of brain stem dysfunction in rhombencephalitis like ataxia, tremor, myoclonic jerks, oculomotor problems (nystagmus, strabismus, or gaze paresis), and bulbar palsy (dysphagia, dysarthria, dysphonia, and facial weakness).

When compared to other viral encephalitis; levels of personality change, rashes, and diarrhea are significantly higher in EV associated encephalitis (EVAE) than in other viruses associated encephalitis. However, studies have shown that neck stiffness is significantly less common in EVAE.[9]

Some cases are mild and reversible but severe cases present with myoclonus followed by the rapid onset of respiratory distress due to neurogenic PE, pulmonary hemorrhage, cyanosis, shock, coma, and apnea and if appropriate interventions are delayed, these will proceed to sudden death.[20,31]

Other systemic complications of enteroviral encephalitis

Enteroviral encephalitis can lead to neonatal sepsis like illness in neonates and viral myocarditis and is associated with some cases of dilated cardiomyopathy.[26,27,30] Destruction of beta cells and acute and chronic inflammatory infiltrates has been reported in islets from cases with CV B infections leading to juvenile onset diabetes mellitus and diabetic keto acidosis following a flu like illness and encephalitis.[28,31]

Diagnosis

Sample for investigation of enteroviral infections include throat, rectal, and ulcer swabs, and samples of serum, urine, CSF, and fluid from vesicle.[29]In enteroviral encephalitis, rectal and throat swabs and serum and CSF samples are required for making a diagnosis.

For enteroviral neurologic disease other than EV 71-associated disease, the sensitivity of EV-PCR of CSF samples is reported to be 76–100%.[31–34] However, CSF viral culture results for patients with EV 71-associated neurologic disease are reported to be positive for only 0–3% of cases.[35] The sensitivity of EV-PCR of CSF samples from patients infected with EV 71 is also poor.[36] The reason that EV 71 is difficult to detect in CSF samples is unclear. Possible explanations for this include the virus only transiently being present in CSF, a lower amount of virus being present in CSF, the EV-PCR assay used not having been optimized for detection of EV 71, and/or different neuropathogenesis manifesting than those exhibited by other EVs.[36]

Species B EVs such as CV A9 and echoviruses B1–6 are usually readily isolated from CSF, unlike species A EVs such as EV 71.[22] A report on the outbreak of viral encephalitis in northern India in 2006 showed that CSF-PCR was positive for EV in 66 (21.6%) of 306 patients. Sequencing and phylogenetic analyzes of PCR products from 59 (89.3%) of 66 specimens showed similarity with EV 9 and EV 76 sequences.[12]

Thus, for making diagnosis of EV encephalitis, samples from throat, rectum, serum, and CSF are taken into consideration and based on the positivity of different isolates; following classification of viral encephalitis has been proposed.

Diagnosis of enteroviral encephalitis is said to be (a) Definitive if (i) virus is detected by PCR or culture in CSF or (ii) by viral detection in both rectal and throat swabs as well as in blood by PCR and absence of other viruses in CSF. (b) Probable encephalitis is labeled if virus is detected in both rectal and throat swabs by PCR and absence of other viruses in CSF. (c) Possible enteroviral infection is labeled when virus is detected in either rectal or throat swab by PCR and absence of other in CSF.[37]

Virus isolation and nucleic acid detection

The gold standard for diagnosis of EV infection per se is virus isolation.[24,30] However, because of poor culture growth and increased sensitivity, specificity and quick turnaround time of molecular reverse transcription-PCR (RT-PCR), nucleic acid detection have become the gold standard for detecting HPeV.[38,39]

Several human and non-human primate cell lines can be used for the culture of EVs. Rhabdomyosarcoma (which is most efficient), human lung fibroblast cells, and African green monkey kidney cells are commonly used culture media. In rhabdomyosarcoma cells, a characteristic cytopathic effect is observed typically 7–10 days after inoculation. Once a cytopathic effect is observed, the virus is identified by neutralization tests in intersecting pools of type-specific antisera or by an indirect immunofluorescence assay with type-specific monoclonal antibodies.[28]

Human parechoviruses grow on tertiary monkey kidney cells, human embryonic lung cell lines, and African green monkey kidney (Vero) cells; however, growth is often poor and may take 14–18 days in some cells.[40]

To detect EVs directly from clinical samples, RNA is first extracted by using various kits available. Pan-EV RT-PCR is then performed by using primers specific for the 5′ untranslated region.[31] Enteroviruses identified by pan-EV RT-PCR are then typed by nucleotide sequencing of the viral protein 1 (VP1) region.[30] To further type enteroviruses, phylogenetic analysis is performed on nucleotide sequences by using available softwares.

In the 1990s, when molecular methods were developed for the diagnosis of EVs in clinical samples, it was found that ECVs 22 and 23 were not detectable using pan-EV PCR primers[37] because of sequence differences between genera[39] Thus, ECVs 22 and 23 were subsequently reclassified as HPeV1 and HPeV2.[41] Presently, more sensitive and specific PCRs have been developed that are capable of detecting all known HPeV types.

Cellular localization

Immunohistochemistry (using virus specific antibodies) and *in situ* hybridization (using virus specific probes) can be used for cellular localization in CNS tissue by direct visualization or microscopy.[16]

Serology

Serological diagnosis of an acute virus infection classically relies on a fourfold increase being shown in the concentrations of a specific neutralizing antibody between the acute and convalescent phases.[28] Although homologous antibodies are produced when young children encounter their first EV infection, heterologous cross-reacting IgG and IgM antibodies are produced by older children and adults following repeated infection with different EV serotypes. The usefulness of this test, therefore, decreases with increasing age.[30]

Neuroimaging

Enteroviruses encephalitis predominantly causes involvement in the following: (1) posterior portion of the medulla oblongata, where the dorsal nuclei of the vagus nerve, the medial longitudinal fasciculus, the reticular formation, and the nuclei of the solitary tract were affected; (2) posterior portion of the pons, where the nuclei of cranial nerves VI, VII, and IX were affected; (3) central portion of the midbrain, where the red nuclei, substantia nigra, and the nuclei of cranial nerves III and IV were affected; (4) bilateral dentate nuclei of the cerebellum; (5) bilateral putamina and thalami, though these were rarely involved; and (6) bilateral ventral horns of cervical spinal cord.[42]

Rhombencephalitis caused by EV 71 shows characteristic lesions in the posterior portions of the medulla oblongata and pons in MRI.[43] Similar findings are found in polio and CV infections; T2-weighted MRI may show hyperintensities in the midbrain and anterior horn of spinal cord. Human parechovirus causes white matter injury. In a study by Verboon-Maciolek *et al.*, mild-to-severe white matter abnormalities were detected in neonates with HPeV meningoencephalitis. Diffuse signal intensity changes of the white matter and punctate white matter lesions, suggestive of petechial hemorrhages, were seen on T1- and T2-weighted spinecho sequences of HPeV meningoencephalitis patients. Increased signal intensity in the corpus callosum, optic radiation, internal capsule, and cerebral peduncle were seen on DWI.[43]

Management

No specific treatment is available for EV. However, certain antiviral drugs and intravenous immunoglobulins have shown improved outcomes in patients with enteroviral encephalitis.

Ribavirin

Ribavirin has been shown to inhibit the replication of a variety of EVs.[16] However, the presence of ribavirin may force the afflicted virus into ''error catastrophe'' by generating a highly variable non-infectious quasispecies swarm and thereby causing lethal mutagenesis.[44] Ribavirin has been found to inhibit both *in vitro* and *in vivo* EV 71 replication. In a study by Li *et al.*, ribavirin-treated mice exhibited decreased mortality, morbidity, and paralysis rates when challenged with EV 71.[45] Ribavirin is in clinical use for other viral infections in humans. Animal trials have shown promising results in EV 71 infections, thus potentiating its future prospect in human enteroviral infections.

Pleconaril

Pleconaril is a novel compound that integrates into the capsid of EVs, preventing the virus from attaching to cellular receptors and uncoating to release RNA into the cell.[46] Pleconaril is able to cross the BBB and remain within the CNS at concentrations that inhibit EV replication.[47] Several studies demonstrate that pleconaril may be a valuable compound in the treatment of EV infections of the CNS.[16] In a study by Rotbart *et al.*,[47] 78% of the patients had a clinical response temporally associated with pleconaril therapy. Patients responded favorably when evaluated for virological, laboratory and radiological responses. Adverse effects were minimal and the drug was generally well-tolerated in this study.[45] In a multicenter, double-blind placebo-controlled study of oral pleconaril by Desmond *et al.*, pleconaril shortened the course of illness compared to placebo recipients, especially in the early disease course.[48] Webster *et al.* have shown the efficacy of pleconaril in treatment of clinically ill patients including immunocompromised individuals.[49] Further trials are required for its use in EV encephalitis.

Intravenous immunoglobulin (IVIG)

During the initial large outbreaks of EV 71 in Asia, IVIG was used by clinicians in Sarawak and Taiwan on the presumptive basis that it would neutralize the virus and have nonspecific anti-inflammatory properties.[50] Analysis of cytokine profiles before and after IVIG treatment showed substantial decreases in concentrations of some proinflammatory cytokines in patients with EV 71 if they had encephalitis with autonomic dysfunction. A prospective quasi-randomized study in a tertiary care center of eastern Uttar Pradesh in 83 consecutive patients of AES complicated by myocarditis found that use of IVIG was associated with decreased mortality and increased left ventricular function.[51] But the major limitation of the study was a low viral yield and lack of follow up data of these patients.

Uncertainty remains; however, over whether this expensive human blood product treatment is really effective, and randomized, placebo-controlled, phase 2 trials are needed.[30]

Newer antiviral drugs

Bovine lactoferrin, pleconaril, shRNA, siRNA, rupintrivir, ribavirin, and 17-AAG have been tested *in vivo*. Ribavirin and amantadine are already in clinical use for other viruses, and rupintrivir and pleconaril are in clinical development.[52]

Supportive therapy

The results of a small, nonrandomised, and retrospective assessment of 24 children with EV 71-induced PE showed that those treated with milrinone had reduced tachycardia and lower mortality than those who did not receive this drug. Peripheral white cell and platelet counts and plasma IL-13 concentrations were also lower, which might indicate an immunomodulatory effect of the drug.[53,54]

Use of large volumes for management of shock with EV 71 outbreaks in Asia, frequently precipitated PE. After it became clear that impaired cardiac function is an important contributor to shock, clinicians were more judicious in their use of intravenous fluids and used inotrope support. Fluid management should, whenever possible, be guided by measurement of central venous pressure.[30] In a meta-analysis of randomized controlled trials for use of hypertonic saline versus mannitol for the treatment of elevated intracranial pressure, hypertonic saline is found to be more effective than mannitol for the treatment of elevated intracranial pressure (ICP).[55] Further large scale randomized controlled trials are required to validate its use in raised ICP due to encephalitis.

Intensive care therapies, including mechanical ventilation, ionotropic support, cardiovascular medications, extracorporeal membrane oxygenation, ventricular assist devices, and heart or liver transplants may be required for severely ill patients.[51,56]

Prognosis

A study in Vietnam concluded that the occurrence of convulsions on admission, the presence of limb weakness, GCS, and age were all significantly associated with fatal outcome, whereas illness day on admission, history of convulsion, and gender were not.[37] The results of a prospective clinical study of nearly 1500 children presenting to one hospital during three EV 71 outbreaks in Sarawak over 7 years showed that neurological involvement was strongly predicted by the presence of at least two of the following: peak temperature of 38.5°C, or more fever for 3 days or longer, and a history of lethargy.[57] Chang *et al.* found that children with cardiopulmonary failure after CNS involvement scored lower on intelligence tests than children with CNS involvement alone.[58]

Enterovirus 71 infection with CNS involvement is associated with long-term neurologic sequelae, delayed neurodevelopment, and reduced cognitive function — conditions that may cause further learning and behavioral problems once children attend school.[58] Common sequelae include focal limb weakness and atrophy, swallowing difficulties requiring nasogastric feeding, central hypoventilation, facial nerve palsies, seizures, and psychomotor retardation. Cerebellar disorders are observed in about 10% of patients after moderately severe brainstem encephalitis, including cranial neuropathies, myoclonus, tremor, and ataxia.[30]

Disclaimer Statements

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