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Neurological Manifestations and Experience with IV Immunoglobulin in Children with Enterovirus 71 Infections

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= Abstract =

Purpose : The objective of the study was to report the neurological manifestations and response to high-dose immunoglobulin (IVIG) therapy in children with enterovirus 71 (EV 71) infections.

Methods : Eleven patients who had the primary clinical marker for enteroviral infection-herpangina or hand-foot-mouth-disease (HFMD) followed by acute neurological manifestations were admitted to Ajou University Hospital from June to September, 2009. All patients were positive for enterovirus (EV) as indicated by reverse transcription polymerase chain reaction (RT-PCR). Among them, EV 71 was confirmed by semi-nested PCR in five patients. All patients received IVIG soon after admission.

Results : The mean age of the patients who were EV 71 positive was 2.5 years (range, 4 months to 5.3 years). Five neurological complications associated with EV 71 infection were identified: meningoencephalitis (3 patients); acute cerebellar ataxia (1 pationt); and complex febrile seizure in another patient. Three patients (60%) had HFMD, and two(40%) had herpangina. None of the patients had neurological sequelae at follow-up.

Conclusion : EV 71 infection should be suspected in young children with epidemic HFMD or herpangina complicated by a variety of neurological manifestations. We have reported the response to high dose IVIG therapy in children with EV 71 infection.

Key Words : Enterovirus 71 infections, Neurologic manifestations, Immunoglobulins, Intravenous

Introduction

Enterovirus (EV) outbreaks are common, although they are newsworthy only when death or disability results from the infection. EV 71 infection has emerged as an important public health problem causing serious neurological manifestations and pulmonary edema/hemorrhage as well as death[1]. Neurological complications associated with Enterovirus 71 (EV 71) are also an emerging infectious disease in Korea because it may progress to life-threatening encephalitis, especially in young children[2, 3].

Many potent EV inhibitors, which act on various targets in viral replication cycles have

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been or are being tested in clinical trials; however, to date, no powerful prophylaxis of EV infection is available. No antiviral agent has been approved by the FDA for treating EVs[4, 5]. Intravenous immunoglobulin (IVIG) has been found to have broad therapeutic applications for the treatment of a variety of infectious and inflammatory diseases. Passive transfer of specific antibodies has been shown to reduce or modify the number of viral infections, including Japanese encephalitis, West Nile virus encephalitis, varicella infection, parvovirus B19 infection and coxsackievirus infection[6, 7]. However, the effects of IVIG on EV 71 have not been previously examined.

Therefore, the presentation and outcome of five children that had neurological involvement with EV 71 infection in Suwon, Korea are reported and we discussed the early treatment with high-dose IVIG.

Methods

Eleven patients had the primary clinical marker for enteroviral infection-herpangina and hand-foot-mouth-disease (HFMD) followed by acute neurological manifestations (7 meningoencephalitis, 1 cerebral ataxia and 3 complex febrile seizures); they were admitted to Ajou university hospital from June to September 2009. All patients were positive for EV by the reverse transcription-polymerase chain reaction (RT-PCR). Among them, EV 71 was confirmed by semi-nested PCR in five patients. The diagnosis of EV 71 infection was obtained from throat swabs, stool specimens, and cerebrospinal fluid. IVIG was administered immediately after admission to prevent progression of the neurological complication symptoms. All the patients received IVIG at a higher dose regimen of 1 g/kg/day for 2 days.

1. Clinical definitions

HFMD is characterized by several days of fever and vomiting; ulcerative lesions of the buccal mucosa, tongue, palate, and gums; and lesions of the hands and feet, which are usually vesicular and occur on the dorsal surfaces, but they may also occur on the palms and soles.

Herpangina is characterized by a small number of lesions in the mouth, particularly the soft palate or tonsillar pillars. The lesions progress initially from red macules to vesicles and finally to ulcerations that can be 2-4 mm in size.

Meningoencephalitis was defined as an illness clinically compatible with the signs of fever, vomiting, headache, and/or definite neurological dysfunction associated with CSF pleocytosis (>5 leukocytes/mL) and with or without parenchymal lesions or substantial meningeal enhancement as identified by brain computed tomography or magnetic resonance imaging.

Acute cerebellar ataxia was defined as the acute-onset of truncal ataxia, with or without other cerebellar signs, after the exclusion of other conditions that might have produced a sudden onset of ataxia.

2. Molecular diagnosis for enterovirus infections and genotyping

All samples were sent to the Korean Center for Disease Control and Prevention (KCDC) to perform the testing. Enteroviral genome detection was attempted on all throat swabs, CSF, and stool specimens collected from patients by real-time RT-PCR using Taqman technology. Briefly, viral RNAs were extracted using the magnetic bead-based viral nucleic acid purifi-

cation protocol described by Boom[8]. Subsequently, one-step real-time RT-PCR was performed using a dual-labeled fluorogenic EV-specific probe and primers designed based on previous data[9]. For genotyping, semi-nested RT- PCR was used to amplify part of the VP1 gene of EV based on the CDC's protocol for the detection of pan-EV and sequencing analysis of the specimens found to be positive by real-time PCR[10].

3. Statistics

Acknowledging a small sample size, we used the Mann-Whitney U test to compare the continuous variables and Fisher's exact test for comparison of the categorical variables. Statistical significance was set at $P<0.05$.

Results

The neurological manifestations of EV 71 infection included meningoencephalitis in 3 patients, acute cerebellar ataxia in 1, and complex febrile seizures in 1 patient. The mean age at the onset of disease was 2.5 years (range, 4 months to 5.3 years). Three of the 5 children were under 3 years of age at the time of onset of EV71 infection (Table 1).

1. Prodromal illness and Exanthem

Five of the children with neurological disease developed a rash prior to/or during their illness. Three patients had HFMD (60%), and two had herpangina (40%). Two children had a skin rash at the time of presentation with the neurological symptoms. The neurological symptoms began one day to two weeks after the onset of skin or mucosal lesions or fever.

2. Clinical Manifestations of Neurologic complications

Three neurologic syndromes were identified on the basis of the extent of neurologic involvement: meningoencephalitis (3 patients), acute cerebellar ataxia (1 patient), and complex febrile seizures (1 patient, Table 1).

1) Meningoencephalitis

Three patients with meningoencephalitis presented with headache, vomiting, fever, and decreased mentality. Two patients had HFMD and one patient had herpangina. All three children recovered fully within two weeks (2-10 days) of admission to the hospital. None had neurological sequelae at follow-up.

2) Acute cerebellar ataxia (case report)

Patient 4 was a 28-month-old boy who was well prior to admission to hospital. The patient

Table 1. Clinical Features of 5 Children with Enterovirus 71 (EV71)-associated Neurological Disease

Patient no	Sex	Age (mo)	Neurological manifestations	Rash	Rash period (day)	Recovery period (day)	MRI findings
1	M	4	Meningoencephalitis	Herpangina	7	4	ND
2	M	4	Meningoencephalitis	HFMD	1	11	ND
3	F	63	Meningoencephalitis	HFMD	3	6	Normal
4	M	28	Acute Cerebellar ataxia	Herpangina	7	9	Normal
5	M	51	Complex febrile seizures	HFMD	14	4	ND

Abbreviations : HFMD; hand, foot, and mouth disease, ND: not done
Rash period: the period from onset of skin rash to the beginning of the neurological symptoms

had suffered from herpangina 1 week ago. At that time he developed mild diarrhea, vomiting and fever which gradually diminished. The child's mother noted that he fell down when attempting to bear weight on his legs. He had a gait disturbance and truncal ataxia. He was eating well, and his mental status seemed normal to the parents. The patient did not seem to be in pain. He had a normal birth history with normal developmental milestones were achieved. On neurological examination, he was moving all extremities freely, with normal strength and sensation. A lumbar puncture was grossly yellowish with final cell counts of 54 WBC and 520 RBC and a negative Gram's stain. Magnetic resonance imaging (MRI) was normal. IVIG was administrated immediately after admission to prevent progression of the symptom, the symptoms improved significantly and resolved completely within 2 weeks. There were no sequelae from the infection at follow-up.

3) Complex febrile seizures

Patient 5 was admitted due to two generalized seizures during the febrile illness (temperature, 40℃) caused by HFMD. The CSF findings were normal in the patient with complex febrile seizures. The mental status of patient was fully alert soon after the cessation of seizures. The patient recovered fully, and no neurological sequelae were identified during follow-up.

Discussion

Enterovirus 71 (EV 71) causes epidemics of hand, foot, and mouth disease associated with neurological complications in young children. The neurological complications of EV 71 infection may occasionally cause permanent paralysis or death. Several large epidemics of severe EV 71 infection in young children, including numerous cases of fatal brain-stem encephalitis, have recently been reported in Asia1[11, 12]. Previously, cases of EV 71 infection associated with neurological complications in Korea were reported by Cho, et al[2] and Moon, et al[3]. In this study, EV 71 was associated with a predominance of meningoencephalitis (in 3 patients), acute cerebellar ataxia (in 1), and complex febrile seizures (in 1). Patient 4 developed acute cerebellar ataxia. Acute cerebellar ataxia (ACA) is a clinical syndrome defined by the rapid onset of cerebellar dysfunction, which manifests primarily as gait disturbance and incoordination. Seen most often in children under 6 years of age, ACA occurs primarily in association with a viral illness and is most commonly described after infection with varicella, Epstein-Barr Virus (EBV), and enterovirus[13]. To our knowledge, this is the first case report of ACA with EV 71 infection in Korea. Seizures that develop during acute enterovirus infections are thought to be mainly febrile seizures[14]. One child (patient 5) in this study presented with generalized seizures associated with fever (temperature, 40℃) and HFMD. He was considered to have had febrile seizures during acute EV 71 infection and to have made a complete recovery.

We could not clearly determine the role of the virus in the pathophysiology of neurological complications associated with EV 71. During the natural course of infection, EV 71 is considered to replicate initially in the enteric or the respiratory mucosa and then to spread to various organs, including the CNS. EV 71 is neurono-tropic, and that, although hematogenous spread cannot be excluded, viral spread into the CNS

could be via neural pathways[15-17]. It has also been postulated that overwhelming virus replication combined with damage in tissues with the induction of toxic inflammatory cytokines is one possible pathogenesis[18]. Immune activation by the EV leads to the production of immunoglobulin M (IgM) type-specific antibodies, which may be detected in the serum 1 week after infection. They are responsible for neutralization and rapid elimination of the virus from the blood and other sites of viral invasion. Most enteroviral infections confer lifelong immunity to the serotype-specific agent. In addition, antibodies to related viruses are known to cross-react, and do so in different patterns, based on the country, serotype, and specific population, making comparisons of disease-based studies amongst these groups difficult[19].

Neurological complications associated with EV 71 are an emerging infectious disease in Korea. In addition, physicians, parents, and caregivers of children have reacted to the infection with panic as a result of media publicity over the local HFMD/herpangina-related deaths in May 2009 in Korea[2, 3, 20]; however, there is currently no effective antiviral therapy available for EV 71 infection. This epidemic prompted the use of IVIG for the treatment of neurological complications associated with enteroviral infections to prevent mortality, although these neurological signs were not fatally complicated. So, in our study IVIG was administered to the patients with neurological complications that presented with characteristic herpangina or HFMD. All of the patients received early IVIG soon after the onset of neurological complications and it appeared to have significant benefit. Infusion of IVIG (1 g/kg/day for 2 days) in the 11 patients was generally well tolerated. However, the effect of IVIG is difficult to evaluate. Clinical indications of IVIG for EV 71 infection are still controversial[21]. Control groups have not readily come by, as official treatment policy recommends IVIG for enterovirus cases with complication. The fast diagnosis of the infection with EV 71 using semi-nested PCR and the subsequent administration of IVIG in a double-blind, randomized, placebo-controlled trial with a larger number of subjects is necessary in order to confirm the efficacy of this strategy.

Antibodies play a key role in the host defense against EV. IVIG is prepared from pools of plasma samples obtained from at least 1,000 healthy donors; thus, IVIG covers numerous antibodies to a variety of viruses that comprise a broad range of immune antibodies directed against pathogens[6, 22-24]. Although the explanation for the mechanism of IVIG action is still lacking, extensive research suggests that IVIG may achieve its therapeutic effects through multiple mechanisms associated with immunomodulation[25]. Wang et al.[26] demonstrated the modulation of cytokine production by IVIG in patients with EV 71-associated brainstem encephalitis.

Patients with neurological complications that present with characteristic herpangina or HFMD are easily suspected as enteroviral (EV) infections. However, there were no clinical markers that may alert physicians to the possibility of this fatal complication and bring about earlier efforts to rescue the afflicted patients before detection of EV 71 infection.

The results of this study, showed that EV 71 infection should be suspected in young children with epidemic HFMD or herpangina complicated by a variety of neurological manifestations and we experienced that early support with high-

dose IVIG therapy might improve the clinical outcome of EV 71 infection. However, the clinical indication and efficacy of IVIG for EV 71 infection remains controversial. In the future, well-designed prospective controlled trials with large numbers must be needed to demonstrate the efficacy of IVIG therapy for severe EV 71 infection.

한 글 요 약

엔테로바이러스 71감염과 관련된 신경학적 증상과 면역글로불린 주사 치료의 경험 보고

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목 적: 본 연구에서는 Enterovirus 71 (EV 71)에 감염된 환아들의 신경학적 증상을 설명하고 고용량 면역글로불린 요법에 대한 경험을 보고하고자 한다.

방 법: 2009년 6월부터 9월까지 포진성구내염(herpangina) 또는 수족구병(hand-foot-mouth-disease)으로 진단된 후 급성 신경학적 증상을 보여 아주대학교 병원 소아청소년과에 입원하였던 11명의 환아를 대상으로 조사하였다. 대상 환아들은 Enterovirus reverse transcription polymerase chain reaction (RT-PCR) 검사상 모두 양성 소견을 보였으며, 이들 중 5명의 환아들에서는 semi-nested PCR 방법으로 EV 71이 확인되었다. 급성 신경학적 증상을 보였던 모든 대상 환아들은 입원 직후 고용량 면역글로불린 요법을 시행 받았다.

결 과: EV 71군의 평균 나이는 2.5세(범위, 4개월-5.3세) 였으며, 3명은 수족구병(60%)을 2명은 포진성구내염(40%)을 보였다. 이들의 급성신경학적 증상은 수막뇌염 3명, 급성 소뇌성 운동실조증과 복합 열성 경련이 각각 1명으로 분류되었다. 입원 직후 고용량 면역글로불린 치료를 받은 환아들은 모두 신경학적 후유증 없이 회복되었다.

결 론: 유행성 수족구병 또는 포진성구내염과 함께 다양한 양상의 신경학적 증상을 보이는 환아들에서 EV 71 감염을 의심해 볼 수 있으며, 대상 환아들의 초기 고용량 면역글로불린 요법이 임상증상의 호전에 기여한 경험을 보고 하고자 한다.

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