Enterovirus 71 Infection and Prevention

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Abstract

Enterovirus 71 (EV71), a single-stranded RNA virus, belongs to family Picornaviridae, genus Enterovirus, species Human Enterovirus A (HEV-A). Most of the infected patients were children, mainly presenting with hand-foot-and-mouth disease or herpangina. In 1998, EV71 epidemic caused 78 deaths, thereafter small-scale epidemics or outbreaks were also noted. EV71 infection is now endemic in Taiwan. Even though EV71 did not result in large epidemics in 2006 and the beginning of 2007, more than one hundred severe cases of EV71 infections had been reported between late 2007 and May 2008, showing that the virus continues to threaten the health of children. The prevention of enterovirus infections had become one of the most important goals in public health policy. This review discusses topics in transmission route, molecular epidemiology, pathogenesis, clinical presentation, prognosis, laboratory diagnosis, therapeutic strategy, and prevention methods, with the objective to serve as a helpful reference for EV71 research and control in the future.

Keyword: Enterovirus 71, Hand-foot-mouth disease, Cardiopulmonary collapse, Sentinel surveillance system

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Foreword

Since Enterovirus 71 (EV71) was identified in 1969 in California, United States, sporadic cases had been reported around the world. Large-scale epidemics resulting in deaths took place in Bulgaria in 1975, Hungary in 1978, Malaysia in 1997, and Taiwan in 1998 and 2001. The virus also caused small-scale outbreaks in Taipei during 1980 and Kaohsiung during 1986. [1].

In 1998, EV71 infection caused 78 deaths, 34 of them were virologically confirmed. In the 2000 and 2001 epidemics, 20 to 30 deaths were reported. Small-scale outbreaks were also noted in 2002, 2003, 2004, and 2005, with less than 10 virologically confirmed deaths reported in each of those years. Most of the infected patients were children, mainly presenting with hand-foot-and-mouth disease (HFMD). Patients with severe diseases often worsened and died within a few days. Pathogenesis of severe disease was central nervous system invasion followed by cardiopulmonary failure [2, 3].

EV71 infection is now endemic in Taiwan. It disappeared in the 2 years following 2005 outbreak, reappeared in southern Taiwan in late 2007 and early 2008. Because the epidemic seems to spread to central and northern part of Taiwan, close monitoring is needed.

The prevention of enterovirus infections had become one of the most important goals in public health policy. In Taiwan, with the knowledge about enterovirus gained through research, proactive prevention had replaced fear.

Virology of Enterovirus 71

EV71, a single-stranded RNA virus, belongs to the family Picornaviridae, genus Enterovirus, species Human Enterovirus A (HEV-A).

Traditionally, enteroviruses encompass polioviruses, echoviruses, and

coxsackieviruses; newer viruses were named with numbers, such as enteroviruses 68 to 71. Since 2000, King et al, classified enteroviruses by genomic sequencing to human poliovirus and human enteroviruses A to D [4]. EV71 belongs to species human enterovirus A.

EV71 was classified into genotypes A, B, and C. Genotype B could be further classified into subtypes B1 to B5; and genotype C into subtypes C1 to C4 [5, 6].

Transmission of Virus

EV71 is primarily transmitted through the fecal-oral route. Droplet transmission might be another route of transmission. During the 1998 epidemic, virus isolation rate was significantly higher from throat swabs than from rectal swabs or feces during acute infection. Possible droplet transmission was also demonstrated in the research conducted by Dr. Chang LY, who identified viruses using reverse transcription polymerase chain reaction in the air collected in the pediatric out-patient clinic and the emergency room.

According to Chung et al, EV71 could survive 1 to 2 weeks in the pharynx and 6 to 8 weeks in feces. Therefore, the transmission of EV71 in the acute phase is probably through droplet transmission; viral load and infectivity are both high at this time. To avoid transmission of disease, infected patients should stay at home and avoid being in school and poorly ventilated places. For the entire 6 to 8 weeks after acute infection, patients should practice good personal hygiene, including frequent hand-washing [7].

High rate of susceptible hosts in population will help the spread of virus. Chang et al found the lowest EV71-seroprevalence rate among children 6 months to 3 years of age, the group with the majority of fatal and severe cases in the

outbreak in 1998. Seropositive rate of anti-EV71 in children over the age of 6 years was similar to the adult population, therefore, fatal or severe cases in this group were seldom seen [8]. Post epidemic seroepidemiological study have shown that EV71-seropositivity rates in areas with large-scale epidemics, such as Taoyuan County, Taichung County, Changhua Country, Ilan County, and Kaohsiung County, were significantly higher than the rate in areas with small-scale epidemics, such as Taipei City and Kaohsiung City.

For susceptible infants and young children, transmissions between siblings in the family and preschool children were probably the major route. Chang et al prospectively analyzed 94 families (433 family members) that had at least one family member hospitalized with EV71 infection. Transmission between siblings or cousins was over 80%, which was higher than that of parents (40%) or other adults (20%). Almost 50% of the infected adults were asymptomatic. When symptomatic, adults usually present with non-specific upper respiratory infections; they rarely have HFMD or herpangina. Infected adults would spread the disease to other members of the family, especially preschool children. Over 90% of the infected children will become symptomatic; 70% will present with HFMD, and 20% will have severe disease [9].

The proportion of susceptible hosts in the population is an important factor in determining the spread of virus and scale of the epidemic. In addition, investigating the effect of geographic region, climate, and ethnicity is also important in understanding viral transmission [8, 10].

Molecular Epidemiology

Gene sequences in the VP1 region, related to the surface protein that bind neutralizing antibody, and the 5'-non-coding region (5'-NCR), related to virus

cloning, are used to analyze the gene evolution or the molecular epidemiology of EV71.

For the 1998 outbreak, phylogenetic analysis of VP1 and 5'-NCR sequence of nine EV71 isolates from patients who died and seven isolates from uncomplicated hand-foot-and-mouth disease patients showed that all but one isolate fell into genotype C. Follow up studies showed that subgenogroup C2 was the major etiologic group in the 1998 outbreak. Subgenogroup B4 became predominant during 1999 to 2003. Subgenogroup C4 emerged and became predominant in 2004. Then subgenogroup B5 became predominant at the end of 2007.

Subgenogroup C4 had been the main genogroup in China since 1998. Identified in 2005 by Mizuta et al, subgenogroup B5 caused outbreaks in Malaysia and Japan [6, 11, 12]. EV71 might spread through international travel. Therefore, subgenogroup C4 might have come from China, then became predominant in Taiwan after 2004 and subgenogroup B5 might have come from Malaysia more recently.

Pathogenesis

The pathogenesis of EV71 infection severe cases warrants further study.

In 1998, pulmonary edema was noted in patients. In addition, severe inflammation of the central nervous system, especially the medulla and cervical nerves, with rapid virus isolation indicated that the central nervous system is the main site of invasion by EV71. This is followed by autonomic dysfunction, andsystemic inflammatory response, with the consequence of cardiopulmonary failure. However, the specific pathogenesis is not clear and it might be multifactorial [13, 14].

Clinical presentation and prognosis

HFMD is the most common presentation in EV71 infections, followed by herpangina. However, coxsackievirus (Cox) A16, A5, A7, A9, A10, B2, B5 may also cause HFMD. Cox A16 and EV71 caused the epidemic of HFMD and herpangina in 1998. Chang et al analyzed 177 cases of EV71 and 64 cases of Cox A16 from Chang Gung Children's Hospital. Vomiting, lethargy, fever > 39°C, fever > 3 days, and having both fever >39°C, fever >3 days were observed more frequently in patients with EV71 infections than those with Cox A16 infections [15].

Most children infected with EV71 recover, only few of them will have central nervous system invasion. Even fewer progressed to cardiopulmonary failure following central nervous system invasion. The central nervous invasion are associated with lethargy, severe vomiting and myoclonic jerks, and cardiopulmonary failure associated with tachycardia, hypertension and hyperglycemia [15, 16].

Chang et al conducted long-term follow-up on 142 children discharged from Chang Gung Children's Hospital and National Taiwan University Hospital, originally diagnosed as severe cases with central nervous system invasion and found:

(1) The best prognosis was noted in patients who had only aseptic meningitis; almost everyone recovered fully.

(2) Of the patients who had encephalitis, encephalomyelitis, or poliomyelitis-like syndrome, even though no cardiopulmonary failure occurred, 20% had sequelae including lower limb weakness or atrophy or facial nerve palsy.

(3) Patients with cardiopulmonary failure had the worst prognosis. Up to 75% had neurologic sequelae including ventilator-dependency, tube feeding, spasms, muscle weakness or atrophy, and facial nerve palsy. Developmental

delays were also found in 20% of the patients. These patients needed long term care and societal support [17].

Laboratory Tests

In March 1999, Taiwan Centers for Diseases Control established laboratory surveillance through13 contract laboratories which test specimens from severe cases under investigation for enteroviruses infection.

Identifying EV71 rapidly and correctly is the most important task and will provide information to doctors treating the patients.

The standard method to identify EV71 is cell culture combined with seroantibody neutralization test. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of EV71 is another sensitive test [18].

Treatment

There are currently no effective antiviral agents EV71. In Taiwan, the Centers for Disease Control recommended a stage -based therapeutic strategy,

Stage 1: Symptomatic treatment is recommended for HFMD, herpangina and non-specific febrile diseases.

Stage 2: When the central nervous system is invaded, such as in encephalitis or meningitis, patients should be hospitalized to restrict fluid intake, control increased intracranial pressure. When seizures occur, anti-epileptic medication should be used. Most importantly, patients should be closely monitored for hypertension, myoclonic jerks, and hyperglycemia. Patients should be transferred to intensive care unit if hyperventilation, falling Glasgow Coma Scale score, respiratory failure, hypertension, or hyperglycemia is noted. There is currently no consensus on the effectiveness of intravenous immunoglobulin (IVIG). In general, IVIG is recommended for patients with encephalitis, encephalomyelitis,

cardiopulmonary failure or poliomyelitis-like syndrome.

Stage 3A: In this stage, there is autonomic dysfunction. There will be elevated blood pressure, tachycardia or pulmonary edema. Intensive care should include continued fluid restriction, frequent monitoring of changes in nervous system and cardiac function. Milrinone or dobutamine could be administered if cardiac output decreases. Intubation with increased positive end-expiratory pressure could be performed when respiratory failure, pulmonary edema, or pulmonary hemorrhage occurs. High frequency oscillator mechanical ventilation might be considered if pulmonary edema and pulmonary hemorrhage worsens.

Stage 3B: In this stage, there is heart failure. Initial presentation is hypotension. Vasopressin should be used immediately to maintain systolic blood pressure. Extra-corporeal membrane oxygenation (ECMO) might be considered when troponin I is >30ng/ml, continued use of high-dose vasopressin, or continuous hypotension with oliguria is noted

Stage 4: This is the recovery stage. Discontinuing vasopressin should be considered. If patient could not be weaned off the ventilator, tracheostomy is recommended. Aggressive chest care may prevent recurrent pneumonia. If neurologic sequelae, like central hypoventilation syndrome, dysphagia, limb weakness or atrophy was noted, consultation with physical therapy specialist is important [19].

Analysis from Chang Gung Children's Hospital found that stage-based management reduced case fatality rate in patients with EV71-related cardiopulmonary failure from 80% to 30%. However, among those who survived, neurologic sequelae increased from 10% to 40% [19].

Pleconaril is a novel antiviral agent against enteroviruses. Studies had shown that it was effective against coxsackieviruses, but not against EV71 [20].

Lactoferrin was shown to inhibit EV71 from entering into human embryonal

rhabdomyosarcoma cells *in vitro*. Recombinant porcine lactoferrin expressed in the milk of transgenic mice protected neonatal mice from a lethal challenge with EV71. However, lactoferrin did not prevent EV71 infections in small scale human trial [21, 22].

Prevention

Prevention is better than treatment. The government, healthcare system, and the general public all need to play its part in preventing EV71 infections.

1. Multilayered surveillance systems were established to include doctors, laboratories and notifiable disease reporting systems which would allow on-going epidemic control and establish disease prevention policy. These include:
   (1) Enhance personal hygiene and promote knowledge of enteroviuses prevention
   (2) Maintain multilayered surveillance systems and educate physicians on the symptoms of severe cases, need for referral to hospitals, and list recommended referral hospitals to achieve the goals of early diagnosis and early treatment.
   (3) Emphasize the maintenance of clinical consultation to elevate quality of medical treatment.
2. In addition to sentinel and laboratory surveillance, nosocomial infection control should be strengthened to include [18, 23]:
   (1) Strengthen medical staff's knowledge of enteroviruses for early diagnosis.
   (2) Practice contact and droplet precaution for in-patients.
   (3) For neonatal infections, enhance education of contacts, changing clothes, washing hands, sterilization and isolation procedures, and to cooperate with public health bureau in epidemic investigation and specimen collection.
3. The general public should pay attention to information of epidemics. Adults and infected children should not be in contact with other children. Children should not share pacifiers or food. Toys should be kept clean. Because the

virus could survive in feces for 6 to 8 weeks, frequent hand-washing could reduce the spread of enteroviruses [18, 23].

4. Enteroviruses could survive in acid or alcohol. To inactivate enteroviruses, aldehyde or bleach is needed. Immersing clothes in hot water and insolating materials under sunlight could reduce the spread of enteroviruses.
5. Development of vaccine is needed to control EV71 infections. Taiwan Centers for Disease Control and the National Health Research Institute are conducting research to develop a vaccine which would cover EV71 and coxsackievirus A16.

Conclusion

Even though EV71 did not result in large epidemics in 2006 and the beginning of 2007, more than one hundred severe cases of EV71 infections had been reported between late 2007 and May 2008, showing that the virus continues to threaten the health of children. In addition to sentinel surveillance, enhanced public health policy, and effective prevention and isolation measures, having continued basic medical science research, improved treatment strategies, and development of vaccine may finally alleviate people's fear of enteroviruses in the near future.

References

1. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. N Engl J Med 1999; 341: 936-42.
2. Wu JM, Wang JN, Tsai YC, et al. Cardiopulmonary manifestations of fulminant enterovirus 71 infection. Pediatrics 2002; 109: E26-.
3. Fu YC, Chi CS, Chiu YT, et al. Cardiac complications of enterovirus rhombencephalitis. Arch Dis Child 2004; 89: 368-73.
4. King AM, Brown F, Christian P, et al. Picornaviridae 2000, In Virus

Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses, pp. 657-78. San Diego: Academic Press.

5. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS Microbiol Reviews 2002; 26: 91-107.
6. Mizuta K, Abiko C, Murata T, et al. Frequent importation of enterovirus 71 from surrounding countries into the local community of Yamagata, Japan, between 1998 and 2003. J Clin Microbiol 2005; 43: 6171-5.
7. Chung PW, Huang YC, Chang LY, et al. Duration of enterovirus shedding in stool. J Microbiol Immunol Infect 2001; 34: 167-70.
8. Chang LY, King CC, Hsu KH, et al. Risk factors of enterovirus 71 infection and associated hand, foot, and mouth disease/herpangina in children during an epidemic in Taiwan. Pediatrics 2002; 109: e88.
9. Chang LY, Tsao KC, Hsia SH, et al. Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. JAMA 2004; 291: 222-7.
10. Li ML, Hsu TA, Chen TC, et al. The 3C protease activity of enterovirus 71 induces human neural cell apoptosis. Virology 2002; 293: 386-95.
11. Shih SR, Ho MS, Lin KH, et al. Genetic analysis of enterovirus 71 isolated from fatal and non-fatal cases of hand, foot and mouth disease during an epidemic in Taiwan, 1998. Virus Res 2000; 68: 127-36.
12. Lin KH, Hwang KP, Ke GM et al. Evolution of EV71 genogroup in Taiwan from 1998 to 2005: an emerging of subgenogroup C4 of EV71. J Med Virol 2006; 78: 254-62.
13. Lin TY, Chang LY, Huang YC, et al. Different proinflammatory reactions in fatal and non-fatal enterovirus 71 infections: implications for early recognition and therapy. Acta Paediatr 2002; 91: 632-5.
14. Wang SM, Lei HY, Huang KJ et al. Pathogenesis of enterovirus 71 brainstem

encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. J Infect Dis 2003; 188: 564-70.

15. Chang LY, Lin TY, Huang YC, et al. Comparison of enterovirus 71 and coxsackie-virus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998. Pediatr Infect Dis J 1999; 18: 1092-6.
16. Chang LY, Lin TY, Hsu KH, et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. Lancet 1999; 354: 1682-6.
17. Chang LY, Huang LM, Gau SS, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. N Engl J Med 2007; 356: 1226-34.
18. Taiwan Centers for Disease Control. Guidelines for the control and prevention of enterovirus infections. March 2008. (In Chinese).
19. Chang LY, Hsia SH, Wu CT, et al. Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2002. Pediatr Infect Dis J 2004; 23: 327-32.
20. Desmond RA, Accortt NA, Talley L, et al. Enteroviral meningitis: natural history and outcome of pleconaril therapy. Antimicrob Agents Chemother 2006; 50: 2409-14.
21. Lin TY, Chu C, Chiu CH, et al. Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells in vitro. J Infect Dis 2002; 186: 1161-4.
22. Chen HL, Wang LC, Chang CH, et al. Recombinant porcine lactoferrin expressed in the milk of transgenic mice protects neonatal mice from a lethal challenge with enterovirus type 71. Vaccine 2008; 26: 891-8.
23. http://www.cdc.gov.tw/sp.asp?xdurl=disease/disease_content.asp&id=1662&mp=1&ctnode=1498