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Clinical analysis of 59 children with hand foot and mouth diseases due to enterovirus EV71 and concomitant viral encephalitis

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Abstract. – OBJECTIVE: To analyzed the clinical features of children with HFMD and viral encephalitis and to summarize some treatments.

PATIENTS AND METHODS: A total of 59 children with HFMD were included in this study. All children underwent complete blood count, blood biochemical test cerebrospinal fluid examination, chest X-ray and brain MRI.

RESULTS: One child died 24 hours after admission due to central respiratory failure with myocardial damage. After the treatment, 58 children had normal temperature, resolved rash, normal complete blood count, biochemical blood tests and cerebrospinal fluid test, respiratory and circulatory symptoms and signs, as well as neurological symptoms, disappeared. The hospitalization time was 12-21 days. After follow-up for 1-3 months, all children were recovered, and without any severe sequelae.

CONCLUSIONS: HFMD and the complicated viral encephalitis usually occurred in the children < 3 years old. The clinical manifestations were not typical. Monitoring of the child's clinical symptoms, signs and relevant examinations was required.

Key Words:
Hand foot and mouth disease, Viral encephalitis, Myocardial damage, Prognosis, Complications.

Introduction

Hand, foot and mouth diseases (HFMD) are a group of diseases caused by enteroviruses in preschool children, especially children < 3 years old. HFMD are characterized by herpes in the skin of hand, foot, mouth and hip[1-3]. The pathogens of HFMD are predominantly Coxsackie virus, Enterovirus 71 (EV71) and ECHO virus. Coxsackie virus A16 type and EV71 are the most common[4,5]. Both the children with HFMD and the children with inapparent infection were the sources of HFMD infection, which could be transmitted through the digestive tract, the respiratory tract, and close contact[6]. The clinical symptoms of most children were mild, including fever, pimples, and herpes on the palms of hand, soles of the feet and hips, and herpes and/or ulcers on the oral mucosa. HFMD could be resolved in most children[7,8]. But a few children could have severe central nervous system damage, leading to aseptic meningitis, encephalitis, and polio-like paralysis, as well as subsequent neurogenic pulmonary edema, and neurogenic myocardial damage. This condition progresses rapidly, with high disability rate and mortalit. This review retrospectively analyzed the clinical data of 59 children with HFMD and viral encephalitis in the Xuzhou Children's Hospital from January 2008 to December 2012. The clinical features and treatment were summarized.

Patients and Methods

Patients

This study included 59 children (30 male and 29 female) and the age of onset was 8 months to 6 years, with a mean age of 21 months. Forty children were < 3 years old, accounting for 71.2%. All these children met the diagnosis criteria of severe case of HFMD as defined by The Outline of Diagnosis and Treatment of HFMD (2008), issued by Office of Ministry of Healthcare[10]: (1) Main manifestations were fever, as well as rash and herpes on the hands, feet, mouth and hips, but in some cases, the symptoms of upper respiratory tract infection were also noted; (2) Some cases had only rash and herpangina; (3) Severe cases had the manifestations of nervous system involvement and respiratory and circulatory failure. Laboratory examination may find increased leukocytes in peripheral blood, hyperglycemia

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and cerebrospinal fluid. Electroencephalogram (EEG), MRI and chest X-ray may show abnormal findings. (4) Based on the clinical diagnosis, the patients who met any of the following criteria were diagnosed as HFMD: positive EV71 nucleic acid; EV71 virus isolated from stool samples; positive anti-EV71 IgM antibody in the patient's serum; the titer of anti-EV71 IgG antibody increased > 4 fold; negative detection of anti-EV71 IgG antibody changed to positive. This study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from all participants before the study.

Clinical Symptoms and Signs

1. The distribution of rash and herpes: (1) Thirty-seven cases of rash and herpes on the hand, foot and mouth, accounting for 62.71% were included in this study. Rash and herpes on the hand were red, small papule and herpes, predominantly on the palm and finger-bending side. Rash and herpes on the foot were red, small papule and herpes, predominantly on the sole of the foot and toe bending side. The herpes and ulcer in the mouth mucosa were red, small herpes and small ulcer, predominantly on the soft palate, hard palate, tongue tip, cheek mucosa, uvula and posterior pharyngeal wall. (2) There were twelve cases of rash and herpes only on the hand and foot, accounting for 20.34%. (3) Nine cases of rash and herpes on the hip, accounted for 15.25%, were red, small papule and red, small herpes, predominantly on the perianal and sacral skin. (4) One case without rash, accounted for 1.69%, had only upper respiratory tract infection, fever and the symptom of nervous system damage.
2. Fever: all 59 children had fever and most of the children had fever 1-2 days before the onset of maculopapule and herpes. The fever persisted for 3-6 days, with irregular patterns. Thirty-seven children accounting for 62.71% had fever > 39°C.
3. The symptoms of respiratory and circulatory system: In the study there were 15 cases of shortness of breath, 10 cases of phlegm rale and moist rale, 16 cases of tachycardia, 4 cases of higher blood pressure, 8 cases of pale face, and 5 cases of cyanosis.
4. Nervous system: 59 cases of listlessness and lethargy, 30 cases of vomiting, 35 cases of headache, 44 cases of hyperarousal (frequent episodes in sleep), 9 cases of paroxysmal irritability, 45 cases of limb shaking, 4 cases of clouding of consciousness and delirium, 9 cases of convulsions, 2 cases of coma, and 22 cases of positive meningeal irritation sign participated in this study.

Laboratory Examinations

All children underwent complete blood count, blood biochemical test (including liver functions, kidney functions, cardiac enzymes, serum ions), cerebrospinal fluid examination, chest X-ray and brain MRI. (1) Complete blood count: 48 children had significantly higher white blood cell count (> 15×10^9/L), accounting for 81.36%; 4 children had significantly higher white blood cell count (< 2×10^9/L), accounting for 6.78%. Both erythrocytes and platelets were normal. Twenty-two children had hyperglycemia (> 9 mmol/L). Nine children had higher cardiac enzyme activities, including 1 child who had significantly higher cardiac enzyme activities: higher CK-MB, CK and LDH activities, and higher troponin I (cTnI) level. C-reactive protein (CRP) level was also higher. (2) Cerebrospinal fluid test: all samples were clear, with higher pressure and white blood cell count (52 cases with predominantly higher monocytes, 7 cases with predominantly multinucleate cells), and normal levels of protein, glucose and chlorides. All samples had negative bacterial culture. (3) Imaging examinations: 48 children had normal cranial MRI, 11 children had partial high cranial MRI. With regard to the chest X-ray, 17 children had patchy consolidation, 30 children had thickened pulmonary markings, 12 children were normal. EEG demonstrated diffused inactive echoes and a few spike and slow waves. EEG indicated 16 cases of sinus tachycardia and 2 cases of Q-T prolongation. (4) Virological examinations: cerebrospinal fluid, throat swab and the serum samples in acute phase were collected for all children and delivered to the Centers for Disease Control and Prevention for virological examinations. The examinations indicated positive EV71 nucleic acid.

Treatment

1. General treatment: water and electrolyte balnce as well as nutrient supply were maintained. Intravenous injection of nutrient and albumin were provided for the children with malnutrition. Medication and physical cooling was provided to the children with ardent fever. Convulsions were managed, anticonvulsants, such as diazepam, phenobarbital, 10% chloral hydrate,

were administered to the children with seizure and convulsion; if these medications did not work, muscle relaxant was administered under controlled mechanical ventilation.

2. Controlled amount of the liquid enhanced oral and skin care: the appropriate amount of the liquid is 50-60 ml/(kg/d)[11]. Herpes on the hands, feet and hips was scrubbed with calamine lotion 2-3 times/day. Children were given timely manicure to prevent scratch at the lesion sites. Hips were maintained clean and dry and hips were cleaned timely after the children urinated or defecated. Oral care was performed with normal saline, the ulcers and herpes were scrubbed with iodine glycerin, and finally, the ulcers and herpes were scrubbed with Smecta or mirabilitum praeparatum paste twice/day and attention was paid to change in the mouth mucosa.
3. Anti-infection treatments: Ribavirin 10-15 mg/kg/d, 2 doses of intravenous infusion in total, for 7 days; or acyclovir, 5-10 mg/kg, intravenous infusion, once / 8 hours, for continuous 10-14 days was used[12]. If preventive antibacterials were necessary for concomitant bacterial infection or severe condition, antibiotics were selected reasonably.
4. Control of the encephaledema and intracranial hypertension by following methods: (1) Control the injection volume of the fluid; (2) Hyperventilation, $PaCO_2$ was controlled at 20-25 kPa; (3) Intravenous injection of dehydrating agent, such as mannitol, i.e. 20% mannitol, 5 ml/kg, fast intravenous injection for 20-30 min, once/4-8 h. Based on the improvement of the condition, the dose was reduced firstly and then the number of administrations. The course of treatment was 4-6 days; Additional furosemide (1-2 mg/kg, iv) and human albumin (1.0 / (kg/d), iv gtt) were administered to the children with severe condition and significant intracranial hypertension.
5. High dose of glucocorticoid: methylprednisolone, 20 mg (kg/d), iv gtt. The dose was reduced based on the condition after continuous administration for 3 days. If the condition was significantly improved, the dose was reduced to 2 mg (kg/d) for 2 days, then to 1 mg (kg/d). Medications were discontinued after 3-5 days; but if the condition was not significantly improved or still severe, the dose was reduced to 10 mg (kg/d), then to 5 mg (kg/d) after 3 days, and this dose was maintained for 3 days until discontinuation.
6. Performed intravenous drip of gamma globulin for 2 days.
7. Monitoring and support of vital signs, and respiratory and circulatory functions: monitoring body temperature, heart rate, respiration, blood pressure, pulse, anal-axillary temperature difference, urine volume, complete blood count, electrolytes, renal function, dynamic monitoring of the changes of glucose and chest X-ray. Trachea cannula was performed for the patients who had concomitant respiratory and circulatory failure, and mechanical ventilation was performed with a respirator. Brain cell nutraceuticals were used in the recovery phase.

Results

In these children, the concomitant diseases included 1 case of neurogenic myocardial damage, 2 cases of central respiratory failure and 4 cases of neurogenic pulmonary edema. As confirmed by virological examinations, all these children had EV71 virus infection and underwent early mechanical ventilation through a respirator with the tracheal cannula. One child died 24 hours after admission due to central respiratory failure with myocardial damage. The other 6 children were successfully rescued. The mean time of mechanical ventilation was 4.5 days, and all respirators had been successfully withdrawn. After the treatment, 58 children had normal temperature, resolved rash, normal complete blood count, blood biochemical tests and cerebrospinal fluid test, respiratory and circulatory symptoms and signs, as well as neurological symptoms, disappeared. The hospitalization time was 12-21 days. After follow-up for 1-3 months, all children were recovered, and without any severe sequelae.

Discussion

Etiology and Epidemiology

Child HFMD is an acute infectious disease due to virus infection. The main pathogens of HFMD are Coxackie virus A group 16, 4, 5, 7, 9, 10 type, B group 2, 5, 13 type, ECHO virus and EV71 in Picornaviridae, Enterovirus genus. EV71 and CoxA16 viruses are main pathogens of HFMD. It was reported that EV71 virus infection accounted for 61.43% of the laboratory examinations, 88% of the severe cases and > 95% of the death cases[13]. HFMD can occur throughout the year,

with the peak incidence during months of June to September. The age of onset is from 24 days to 17 years, and the children from 6 months to 3 years account for 82.54%, and the children from countryside account for 80.68%. It was reported that severe cases of HFMD accounted for 0.2% of the total cases, the severe cases in 2009 and the first half of 2010 accounted for 0.95% and 1.57%, respectively[14,15]. Severe cases, especially critical cases, were predominantly caused by EV71 virus infection. It was reported that 18.6% of the EV71-infected children had neurological complications in Taiwan and China. The mortality of HFMD children has been increasing every year, from 0.2% in 2007 to 0.31% in 2009 and 0.55% in 2010 (until July 31st). This does not only reflect the fact that the enlarged prevalent area of EV71 virus infection results in increased severe cases, but also indicates that this trend is associated with enhanced recognition of HFMD by physicians and improved report system.

Clinical Characteristics

All HFMD children had typical rash and fever to some extent, which may persist for 1-7 days. These symptoms usually persisted for 1-5 days, or even 10 days, with a mean duration of 5 days; some children may have headache, dizziness, vomiting and lassitude, and children with severe infection may have convulsion with limb numbness, viral encephalitis, and viral myocarditis. A few children died of encephalitis and myocarditis. Fever, anorexia, and lassitude occurred earlier, rash occurred 1-2 days later, including hand, foot and mouth blisters, hip and lower limb papule and oral ulcer. The rash was characteristic of solid papule and usually occurred locally. A severe rash may extend systemically and predominantly to palmar surface. The number of ovals or circular rash or herpes varied. Inflammatory flush occurred around the rash, with little intra-blister liquid. The blisters were deep; the wall was not easy to be broken; some scab, hip and lower limb papule, herpes and small ulcer on the oral mucosa may occur with a runny nose and loss of appetite[16]. HFMD could involve central nervous system in the children of all ages; the prevalence was highest in the children from 6 months to 2 years, with 70% of total prevalence in the children < 3 years.

According to Chinese Center for Disease Control and Prevention (CDC), 95% of the severe cases and death cases were < 3 years old. The morbidity in the children > 5 years was negatively related to the age. The seroepidemiological study indicated that the neonate < 6 months may have less infection due to the antibodies from the mother, whereas the morbidity in the children > 6 months was the same with that of general population[17]. The clinical manifestation could be aseptic meningitis, encephalitis, cerebellitis, poliomyelitis-like syndrome, and encephalomyelitis when central nervous system was involved in severe HFMD. During 2-4 days in the course of HFMD, neurological symptoms should be monitored closely, including the common symptoms of severe HFMD with encephalitis, such as limb jitter, lassitude, irritability, vomiting, and amyosthenia. The neurotoxic effect of EV71 could cause severe complications in the infected patients. The mechanism of action may be that EV71 could induce the change of cellular immunity, such as a significant decreased T-lymphocyte level, stimulate the increased level of pro-inflammatory cytokines, induce nerve cell apoptosis, and cause severe neurological complications through the binding of receptor and virus antigen[18]. The HFMD due to EV71 virus infection was characteristic of central nervous system disorder, and predominant in infants and children. EV71 mainly induced inflammation in central nervous tissue (encephalitis, aseptic meningitis, and brain stem encephalitis), which resulted in nervous system disorder. The lesion was most common in cerebral cortex, brain stem, and spinal cord, but less common in cerebellar cortex, thalamus nucleus, peripheral nerves and autonomic ganglia[19]. In this group, all children had lassitude, drowsiness, irritability, limb jitter, amyosthenia, unstable walking, and vomiting. Some children had seizure, myoclonus, nystagmus, flaccid paralysis or coma. Four children had neurogenic pulmonary edema, 2 children had respiratory and circulatory failure, and one child had neurogenic myocardial damage.

Diagnosis and Treatment

Diagnosis

Risk Factors

A large number of studies showed that child (especially < 3 years of age) was at a higher risk of severe cases, this may be related to the immaturely developed organs and tissues and weak resistibility in children. Since the prevalence of

HFMD in 2006, most studies found that this disease could progress into severe disease in a short time, especially in the children < 3 years: (1) persistent high fever; (2) lassitude, vomiting, limb myoclonus, amyosthenia, and convulsion; (3) increased breathing rate and heart rate; (4) cold sweat, poor peripheral circulation; (5) hypertension or hypotension; (6) significantly higher peripheral leukocyte count; and (7) hyperglycemia. But a controversy exists about the prediction criteria of significantly higher peripheral leukocyte count and hyperglycemia.

Clinical Diagnosis

Common patient had rash at the hand, foot, mouth and hip, with or without fever. Severe patients may have the involvement of nervous system and respiratory and circulatory disorders. Laboratory examinations indicated increased peripheral leukocyte count and glucose level, and abnormality in CSF fluid examination, chest X-ray, EEG, echocardiography and brain and spinal cord MRI. Very few severe patients did not have concomitant typical rash. The diagnosis of these patients was very difficult and it was only established in combination with corresponding laboratory examinations. In this study, one child had no rash, but only upper respiratory infection, fever, and neurological damage. The serum sample in acute phase was collected for virological test, which indicated positive EV71-specific nucleic acid. Therefore, for those patients without rash, the diagnosis of HFMD must be established carefully.

Laboratory Diagnosis

Virus isolation was the gold standard for clinical diagnosis of viral diseases. Common specimen for the isolation of virus included throat swab or lotion, stool or anal swab, herpes liquid, brain, lung, spleen, and lymph node. If the patient had neurological symptoms, CSF specimen could be collected. The patient who met any of the following criteria could be confirmed[20]: (1) human enterovirus (the viruses that are proved to cause HFMD, such as CAV16, EV71) could be isolated from throat swab, anal swab, stool, throat lotion, CSF, serum, herpes liquid, spleen, lung, brain, lymph nodes; (2) EV71 and CAV16 specific nucleic acid could be detected from throat swab, anal swab, stool and throat lotion, or human enterovirus specific nucleic acid could be detected from herpes liquid, CSF, serum, spleen, lung, brain, lymph node; (3) The titer of human enterovirus specific neutralizing antibody in the serum specimen is higher than 1/256, or the titer in recovery phase is > 4 fold higher than that in acute phase.

Treatment

HFMD is a disease due to viral infection, and no effective anti-virus drugs are yet available. There has been no effective therapy for severe HFMD, for which the treatment principle is early prevention and symptomatic treatment. A large number of studies showed that the effective therapy for severe HFMD was the combination treatment that predominantly used high dose of hormone, intravenous injection of gamma globulin (IVIG) for pulse therapy, respiratory and circulatory support, lowering the intracranial pressure by dehydration, on the basis of active defervescence and anti-virus treatment[21]. When HFMD is complicated with viral encephalitis, a combination treatment should be used, including anti-infection treatment, symptomatic treatment, maintaining the functions of relevant organs and preventing the complications, etc. The main treatment included: (1) Lowering the intracranial pressure by dehydration; (2) the pulse therapy of glucocorticoids; (3) the pulse therapy of gamma globulins; (4) mechanical ventilation[22-24].

Neurogenic pulmonary edema was an acute edema resulting from sudden increased intracranial pressure due to central nervous system damage. It was characteristic of rapid onset, severe condition, difficult treatment, and a mortality rate of 90%. It is important to recognize the early manifestation of neurogenic pulmonary edema, perform active mechanical ventilation, especially positive end-expiratory pressure (PEEP) that could reduce pulmonary infiltration, prevent pulmonary edema and pulmonary hemorrhage, as well as improve pulmonary ventilation, and increase oxygen saturation so as to improve the condition and decrease the mortality.

Prevention

Although HFMD is a common infectious but preventable disease in China and globally, a few sporadic cases were found in this city (Xuzhou)[25,26]. HFMD can be prevented if following habits are practiced: frequent hand-washing, drinking boiled water, eating cooked food, no contaminated, raw or unclean food should be consumed; open windows for ventilation; frequent sunbath; timely treatment on the onset

of disease[27]. By providing active treatment to HFMD children, effective nursing should also be provided as well as the cooperation of parents should be obtained. Health education, including popular prevention and healthcare, especially the fact that hand hygiene was an important aspect of improving the prevention and treatment efficacy of HFMD, should be elaborated to the children and parents[28-30]. For the suspicious cases, timely pathogenic examination should be performed to diagnose, provide treatment and improve the patient's prognosis, meanwhile, perform epidemiological monitoring and quarantine inspection. Mild, severe or critical treatment should be provided. Strict diagnosis, screening, and rescue for critical patients should be performed.

Conclusions

HFMD and the complication viral encephalitis usually occurred in the children < 3 years old; and the clinical manifestations were not typical. Children have poor expression, thus closely monitoring of the child's clinical symptoms, signs should be performed, and relevant examinations should be completed. Our hospital undertook the task of treatment and reported the severe and critical cases of HFMD in Huaihai Economic Zone. We concluded the following key points through clinical practice: (1) Early detection, early diagnosis, and active treatment were important to the prognosis; (2) The children with viral encephalitis should be administered with high dose of glucocorticoids, intravenous injection of gamma globulins for pulse therapy, and lowering the intracranial pressure by dehydration; (3) The indication of mechanical ventilation should be extended.

Thus, HFMD was the cause of high morbidity in children, especially severe HFMD, which threatened the children's health and had high mortality rate. Currently, the researchers and physicians suggest further investigation in the following aspects: (1) the study of HFMD vaccine; (2) the discovery of specific and effective anti-virus drugs; and (3) early prevention and standardized treatment of severe cases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Beh PL, Farid AJ, Nabil SH, Putri Y, Zamberi S. Detection and characterization of viruses causing hand, foot and mouth disease from children in Seri Kembangan, Malaysia. Trop Biomed 2014; 31: 654-662.
2) Agrawal R, Bhan K, Balaggan K, Lee RW, Pavesio CE, Addison PK. Unilateral acute maculopathy associated with adult onset hand, foot and mouth disease: case report and review of literature. J Ophthalmic Inflamm Infect 2015; 5: 2.
3) Apalla Z, Sotiriou E, Pikou O, Lefaki I, Lallas A, Lazaridou E, Ioannides D. Onychomadesis after hand-foot-and-mouth disease outbreak in northern Greece: case series and brief review of the literature. Int J Dermatol 2015; 54: 1039-1044.
4) Liao Y, Chen KH, Dong XM, Fang Y, Li WG, Huang GY, Song W. A role of pre-mir-10a coding region variant in host susceptibility to coxsackie virus-induced myocarditis. Eur Rev Med Pharmacol Sci 2015; 19: 3500-3507.
5) Bruning AH, Sanden SM, Hoedt AE, Wolthers KC, Kaam AH, Pajkrt D. An atypical course of coxsackievirus A6 associated hand, foot and mouth disease in extremely low birth weight preterm twins. J Clin Virol 2015; 65: 20-22.
6) Jacobsen L, Zimmerman S, Lohr J. Nail Findings in Hand-Foot-and-Mouth Disease. Pediatr Infect Dis J 2015; 34: 449-450.
7) Zhu Y, Jiang Z, Xiao G, Cheng S, Wen Y, Wan C. Circadian rhythm disruption was observed in hand, foot, and mouth disease patients. Medicine (Baltimore) 2015; 94: e601.
8) Ji P, Chen C, Hu Y, Zhan Z, Pan W, Li R, Li E, Ge HM, Yang G. Antiviral activity of Paulownia tomentosa against Enterovirus 71 of hand, foot, and mouth disease. Biol Pharm Bull 2015; 38: 1-6.
9) Long L, Xu L, Xiao Z, Hu S, Luo R, Wang H, Lu X, Xu Z, Yao X, Zhou L, Long H, Gong J, Song Y, Zhao L, Luo K, Zhang M, Feng L, Yang L, Sheng X, Fan X, Xiao B. Neurological complications and risk factors of cardiopulmonary failure of EV-A71-related hand, foot and mouth disease. Sci Rep 2016; 6: 23444.
10) Teo FM, Chu JJ. Diagnosis of human enteroviruses that cause hand, foot and mouth disease. Expert Rev Anti Infect Ther 2016; 14: 443-445.
11) Deng HL, Zhang YF, Li YP, Zhang Y, Xie Y, Wang J, Wang XY, Dang SS. N-terminal pro-brain natriuretic peptide levels associated with severe hand, foot and mouth disease. BMC Infect Dis 2016; 16: 585.
12) Shen XM, Wang WP. Pediatrics. 7th Edition. Beijing: People's Medical Publishing House, 2008; pp. 406-407.
13) Long DL, Zhu SY, Li CZ, Chen CY, Du WT, Wang X. Late-onset nail changes associated with hand, foot, and mouth disease: a clinical analysis of 56 cases. Pediatr Dermatol 2016; 33: 424-428.

14) Ministry of Health, People's Republic of China. The notifiable diseases in China in 2009 and 2010 January. http: //www. moh. gov. cn. public files business /htmlfiles /mohbgt /s3582 /201002 /46043.

15) Ministry of Health, People's Republic of China. Press briefing on the prevention and control of HFMD.

16) Lin H, Sun L, Lin J, He J, Deng A, Kang M, Zeng H, Ma W, Zhang Y. Protective effect of exclusive breastfeeding against hand, foot and mouth disease. BMC Infect Dis 2014; 14: 645.

17) Liu H, Pan JH. The clinical characteristics of EV71 infection and the advance of the treatment. Anhui Medicine 2008; 29: 372-373.

18) Gao N, Dang SS, Li M. The advance of the pathogenesis mechanism of the neurological disease due to EV71-caused HFMD. Chinese Journal of Experimental and Clinical Infectious Diseases 2012; 6: 162-164.

19) Wong KT, Munisamy B, Ong KC, Kojima H, Noriyo N, Chua KB, Ong BB, Nagashima K. The distribution of inflammation and virus in human enterovirus 71 encephalomyelitis suggests possible viral spread by neural pathways. J Neuropath Exp Neur 2008; 67: 162-169.

20) Huang LF. The advance of the diagnosis and treatment of HFMD children. Chinese Basic Medicine and Pharmacy 2012; 19: 2050-2051.

21) Xiong XY, Liu JF. The advance of severe HFMD. Int J Pediatr 2011; 38: 487-490.

22) Feng BF, Zhu KR. Serum levels of ferritin and neuron-specific enolase in children with hand-foot-mouth disease complicated by acute viral encephalitis. Zhongguo Dang Dai Er Ke Za Zhi 2012; 14: 515-517.

23) Chang LY, Lee CY, Kao CL, Fang TY, Lu CY, Lee PI, Huang LM. Hand, foot and mouth disease complicated with central nervous system involvement in Taiwan in 1980-1981. J Formos Med Assoc 2007; 106: 173-176.

24) Liu Chunfeng, Xiong Xiaoyu. The advance of the diagnosis and treatment of the children with severe HFMD. J Appl Clin Pediatr 2012; 27: 1377-1380.

25) Xie YH, Chongsuvivatwong V, Tan Y, Tang ZZ, Sornsrivichai V, McNeil EB. Important roles of public playgrounds in the transmission of hand, foot, and mouth disease. Epidemiol Infect 2015; 143: 1432-1441.

26) Lv X, Qiu M, Chen D, Zheng N, Jin Y, Wu Z. Apigenin inhibits enterovirus 71 replication through suppressing viral IRES activity and modulating cellular JNK pathway. Antiviral Res 2014; 109: 30-41.

27) Cai Y, Ku Z, Liu Q, Leng Q, Huang Z. A combination vaccine comprising of inactivated enterovirus 71 and coxsackievirus A16 elicits balanced protective immunity against both viruses. Vaccine 2014; 32: 2406-2412.

28) Song Y, Wang F, Wang B, Tao S, Zhang H, Liu S, Ramirez O, Zeng Q. Time series analyses of hand, foot and mouth disease integrating weather variables. PLoS One 2015; 10: e0117296.

29) Zhao YY, Jin H, Zhang XF, Wang B. Case-fatality of hand, foot and mouth disease associated with EV71: a systematic review and meta-analysis. Epidemiol Infect 2015; 143: 3094-3102.

30) Song C, Yibing C, Guo Y, Jin Z, Cui Y, Gu X. Risk factors of severe hand, foot and mouth disease complicated with cardiopulmonary collapse. Infect Dis (Lond) 2015; 47: 453-457.