

Clinical features of severe cases of hand, foot and mouth disease with EV71 virus infection in China

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Abstract

Introduction: Hand, foot and mouth disease (HFMD) caused by EV71 infection has become one of the major public health issues in China, which deeply affects children's health. The prevention and control of EV71 is a challenge currently because there is no safe and effective vaccine or antiviral medications available.

Material and methods: A case control study was conducted in a designated hospital to compare severe and mild cases of patients infected with the EV71 virus. Demographic information along with clinical features of HFMD was collected through a standardized questionnaire. Multi-factorial logistic regression was used to analyze independent associations between potential risk factors and severe HFMD.

Results: There were 120 cases (60 cases and 60 controls) collected. The male-to-female ratio was 1.3 : 1 in the case group and 1.7 : 1 in the control group. Multi-factorial logistic regression revealed that the main risk factors for severe cases were highest body temperature being $\geq 38.5^{\circ}\text{C}$ (OR = 9.45, 95% CI: 2.07–43.11, $p < 0.05$), first visited a village level clinic (OR = 4.72, 95% CI: 1.15–19.45, $p < 0.05$), etc.

Conclusions: Close surveillance combined with laboratory testing should be in place during the epidemic period of HFMD. Grass root level medical facilities and training of clinical and laboratory staff should be reinforced so that the diagnostic and treatment capacity can be improved.

Key words: EV71, hand, foot and mouth disease, risk factor.

Introduction

Hand, foot and mouth disease (HFMD) is an infectious disease commonly seen in children that is caused by a variety of human enteroviruses. Most cases occur in preschool children, with the highest incidence observed in children ≤ 3 years old [1]. The main enteroviruses causing HFMD include enterovirus 71 (EV71), coxsackievirus A16 (CA16) and some serum types of echovirus, among which EV71 infection can cause high incidence of severe cases and death. The main causes of death

are severe brainstem encephalitis and neurogenic pulmonary edema (NPE) [1], and the diseases progress very quickly, making early detection difficult. The patients and the people with latent infection are all sources of infection, and the most important transmission routes of infection are the digestive tract, respiratory tract, close contact, etc [2]. The disease is characterized by fever and maculopapules or herpes appearing at the sites of hand, foot, mouth and other places. Commonly, the disease is mild and self-limiting. The majority of cases fully recover within 1 week, with a few cases presenting with meningitis, encephalitis, encephalomyelitis, pulmonary edema, circulatory disturbance, etc [1].

EV71 was first identified in 1969, when the epidemic of infantile encephalitis and aseptic meningitis occurred in the state of California until 1972, when Schmidt *et al.*, for the first time, separated EV71 in the stool specimens from patients with severe central nervous system diseases (including meningitis, encephalitis and acute flaccid paralysis (AFP)) and reported it first in 1974 [3]. Since then, many countries and regions have reported the infection and epidemiology of EV71 [4–14]. At the end of the 1990s, HFMD caused by EV71 infection had become a pandemic in many countries of Asian-Pacific regions, with an increasing number of patients dying from central nervous system complications [9–11, 13, 14]. The HFMD caused by EV71 infection has become one of the major public health issues in China [12–14], which mainly affects children's health, and consequently affects the society as a whole. The prevention and control of EV71 is a challenge because there is no safe and effective vaccine or antiviral medications available.

This study analyzed the risk factors for children with severe HFMD caused by the EV71 virus, to explore how to early detect the severe cases, so as to reduce the occurrence of severe cases and provide evidence-based information for the prevention and control of HFMD.

Material and methods

Study samples

A case-control study was conducted from March to July, 2009 in Shangqiu City, Henan Province, China. The ethical committee of the Chinese Center for Disease Control and Prevention (Institutional Review Board) approved the study protocol. Anonymity and confidentiality were guaranteed to the participants, and oral informed consent from participants' parents/guardians was required during the survey because the participants were children under 4 years old. The ethics committee approved that procedure and required the interviewers to document the contact information of the partic-

ipants' parents/guardians, e.g. phone number, for any further needs. A total of 120 HFMD cases (60 severe cases as the case group and 60 mild cases as the control group) caused by EV71 infection were collected from a designated hospital which had established a special unit for HFMD.

Case definition

Refer to the relevant regulations set in the "Guideline of diagnosis and treatment of enterovirus 71 (EV71) infection (2008 edition)" [15].

- **Severe cases:** Progression of disease that involves the nervous system, with symptoms of respiratory and circulatory disturbance; laboratory testing reveals elevated leukocyte count in peripheral blood, abnormal cerebrospinal fluid, elevated level of blood glucose, and abnormalities can sometimes be found in electroencephalograph (EEG), cerebrospinal magnetic resonance imaging (MRI), chest X-ray, and echocardiogram.
- **Mild cases:** Fever accompanied by rash appearing at the sites of hand, foot, mouth and buttock, with a few cases not showing fever.

The case group consists of inpatients with severe HFMD caused by EV71 infection, which was confirmed by laboratory testing in the designated hospitals for receiving and treatment of HFMD patients, while the control group consists of outpatients seeking treatment at the same hospital during the same period, who were confirmed by laboratory testing to be mild HFMD patients with EV71 infection.

The standardized questionnaire was designed by public health professionals and revised by clinical experts on HFMD and epidemiologists. After the pilot study, it was used by trained epidemiological investigators to survey the parents or guardians of the children recruited into the study; demographic information along with clinical features of HFMD, such as clinical presentation, treating process, close contact history and previous disease history of the children was collected. Five percent of the eligible questionnaires were sampled for verification, by referencing core indicators in the instructions for completing the risk factor questionnaire, and to timely correct any missing parts or logic errors, in order to ensure the completeness and reliability of the data collected.

Laboratory testing

Real time RT-PCR was used to test the specimen collected from the patients with HFMD. Throat swab and stool samples were collected within 3 days of disease occurrence, and specific primers tested by nucleic acid of human enterovirus, CA16 and EV71 were used to perform RT-PCR on the specimen to determine the serotype of the virus.

Statistical analysis

Epi data 3.2 was used to build a database, Epi Data 3.1 (Epi Data for Windows; Epi Data Association, Odense, Denmark) was used to establish a database by double entry; and SAS 9.2 software (SAS Institute) was used to perform data analysis. The χ^2 test, *t* test and rank sum test were used for univariate analysis; and for the significant variables, non-conditional logistic regression was used for multi-factorial analysis. The backward method was used for screening of variables, and goodness-of-fit tests (Hosmer-Lemeshow) were performed on the logistic model.

Results

Basic information

A total of 120 HFMD cases (60 severe and 60 mild) caused by the EV71 virus were collected for this study. The patients had a median age of 18 months (range: 2–43 months) for the case

group and 17 months (range: 1–46 months) for the control group. For the age distribution of samples please see Table I. The male-female ratio was 1.3 : 1 in the case group and 1.7 : 1 in the control group, and the χ^2 test revealed no statistically significant difference in sex ratio between the two groups ($\chi^2 = 0.5509$, $p = 0.4579$). The χ^2 test showed no significant difference between the two groups in delivery method, guardian, and place of residence, and the two groups are comparable (Table II).

Univariate analysis

Univariate analysis on the onset of disease and hospital visiting process found that 17 variables were associated with severe HFMD when setting the significance level at $p = 0.05$. The results showed that risk factors for severe HFMD include fever occurring in the whole disease course (OR = 4.67, 95% CI: 1.45–15.05), fever being the first symptom (OR = 9.04, 95% CI: 3.95–20.68), the

Table I. Distribution of age between the two groups

Age group [months]	Case group		Control group	
	Number	Ratio (%)	Number	Ratio (%)
0–	3	5.00	2	3.33
6–	10	16.67	8	13.33
12–	22	36.67	25	41.67
18–	13	21.67	8	13.33
24–	3	5.00	5	8.33
30–	3	5.00	4	6.67
36–	5	8.33	6	10.00
42–	1	1.67	2	3.33
Total	60	100	60	100

Table II. Comparison of basic information between case and control group

Variable	Case group	Control group	χ^2	Value of p
Sex	Female	26 (43.33)	22 (36.67)	0.55
	Male	34 (56.67)	38 (63.33)	
Delivery method	C-section	15 (25.00)	14 (23.33)	0.07
	Natural	45 (75.00)	46 (76.67)	
Guardian	Parent	12 (20.00)	15 (25.00)	0.4265
	Grandparent	48 (80.00)	45 (75.00)	
Place of residence	Rural	53 (88.33)	49 (81.67)	1.26
	Rural-urban continuum	3 (5.00)	6 (10.00)	
	Urban	4 (6.67)	5 (8.33)	

Table III. Univariate analysis on the risk factors for severe HFMD

Variable		Case group	Control group	OR value (95% CI)	Value of <i>p</i>
Population classification	Scatter-lived children	58	51	1.00	0.0274
	Kindergarten children	2	9	0.20 (0.04–0.95)	
Fever	No	4	15	1.00	0.0062
	Yes	56	45	4.67 (1.45–15.05)	
First symptom	Fever and rash	3	1	1.00	
	Fever first	44	14	9.04 (3.95–20.68)	0.0001
	Rash first	13	45	0.09 (0.04–0.22)	0.0001
Highest body temp. $\geq 38.5^{\circ}\text{C}$	No	14	28	1.00	0.0002
	Yes	42	17	4.94 (2.10–11.60)	
Rash	No	9	1	1.00	0.0092
	Yes	51	59	0.10 (0.01–0.80)	
Macula	No	33	50	1.00	0.0153
	Yes	18	9	3.03 (1.22–7.55)	
Herpes appearing at isthmus of fauces	No	26	45	1.00	0.0059
	Yes	25	14	3.09 (1.37–6.97)	
Vomiting	No	33	51	1.00	0.0004
	Yes	27	9	4.64 (1.94–11.09)	
Convulsions	No	33	52	1.00	0.0001
	Yes	27	8	5.32 (2.16–13.10)	
Lethargy	No	40	52	1.00	0.0099
	Yes	20	8	3.25 (1.30–8.14)	
Self-medication before hospital visit	No	30	48	1.00	0.0006
	Yes	30	12	4.00 (1.78–8.99)	
Take antipyretic before visit	No	2	4	1.00	0.0003
	Yes	26	8	4.97 (2.02–12.26)	
Visited village doctor	No	26	42	1.00	0.0033
	Yes	34	18	3.05 (1.44–6.47)	
Level of clinic visited first	Above county level	14	24	1.00	
	Township level	12	19	0.54 (0.23–1.24)	0.1471
	Village level	34	17	3.31 (1.55–7.07)	0.0020
Diagnosed or not during first visit	No	33	19	1.00	0.0102
	Yes	27	41	0.38 (0.18–0.80)	
Contact with HFMD children	No	34	46	1.00	0.0368
	Yes	26	14	2.32 (1.05–5.13)	

highest body temperature $\geq 38^{\circ}\text{C}$ ($\text{OR} = 4.94$, 95% CI: 2.10–11.61), self-medication before hospital visit ($\text{OR} = 4.00$, 95% CI: 1.78–8.99), first visited a village level clinic ($\text{OR} = 3.31$, 95% CI: 1.55–7.07), visited a village doctor ($\text{OR} = 3.05$, 95% CI: 1.44–6.47), and contact with children with the disease ($\text{OR} = 2.32$, 95% CI: 1.05–5.13) (Table III).

Multi-factorial analysis

For the significant variables found during univariate analysis, a non-conditional logistic regression model was used for multi-factorial analysis.

The backward method was used for screening of variables. When $\alpha = 0.05$, 7 variables were introduced into the model; they were fever being the first symptom ($\text{OR} = 5.85$, 95% CI: 1.58–21.67), the highest body temperature $\geq 38.5^{\circ}\text{C}$ ($\text{OR} = 9.45$, 95% CI: 2.07–43.11), rash type being macula ($\text{OR} = 8.12$, 95% CI: 1.50–43.94), herpes appearing at isthmus of fauces ($\text{OR} = 8.08$, 95% CI: 1.59–40.99), lethargy ($\text{OR} = 10.96$, 95% CI: 1.85–64.85), first visited a village level clinic ($\text{OR} = 4.72$, 95% CI: 1.15–19.45), and contact with children with the disease ($\text{OR} = 8.14$, 95% CI: 1.66–39.97). Regres-

Table IV. Multi-factorial non-conditional logistic regression on risk factors for severe HFMD

Variable	β	S.E.	Wald χ^2 value	Value of p	OR value (95% CI)
Constant	-5.28	1.20	19.24	0.0001	
First symptom is fever	1.77	0.67	6.99	0.0082	5.85 (1.58–21.67)
Highest body temp. $\geq 38.5^\circ\text{C}$	2.25	0.77	8.41	0.0037	9.45 (2.07–43.11)
Rash type is macula	2.09	0.86	5.91	0.0150	8.12 (1.50–43.94)
Herpes appearing at isthmus of fauces	2.09	0.83	6.37	0.0116	8.08 (1.59–40.99)
Lethargy	2.39	0.91	6.96	0.0083	10.96 (1.85–64.85)
First visited village level clinic	1.55	0.72	4.62	0.0316	4.72 (1.15–19.45)
Contact with HFMD children	2.10	0.81	6.67	0.0098	8.14 (1.66–39.97)

sion coefficients for the 7 variables were all statistically significant ($p < 0.05$) (Table IV).

Discussion

The general population is vulnerable to EV71 infection, especially children under 5 years old. In children less than 3 years old, the infection can more easily cause severe nervous system diseases and death in severe cases [9–11, 13, 14]. This study showed that the likelihood of getting HFMD for kindergarten-age children is 0.20 times that for live-scattered children (the children did not go to Kindergarten live in their own home). A serum epidemiological study [16] showed that about 50% of newborns have got a neutralizing antibody of EV71 from their mothers, whose amount decreased quickly after 1 month. For children of 1–23 months old, the positive rate of EV71 antibody is as low as 0.80%, for children between 2 and 5 years old, the rate increases by 12% each year, and it reaches a stable state of 50–70% when above 5 years old. Therefore, the target populations of EV71 are mostly children under 5 years old, with children aged 1–3 years accounting for a large part, who often live scattered.

The HFMD is mainly characterized by fever, and rash and herpes appearing at the sites of hand, foot and mouth, with most cases presenting with mild symptoms. However, a few cases, especially children infected with EV71, can present with meningitis, encephalitis, encephalomyelitis, neurogenic pulmonary edema, circulatory disturbance and other symptoms. It is likely to progress very quickly for a few severe cases and death or sequelae could occur [18, 19]. In the EV71 epidemic in Taiwan in 1998, most HFMD children presented first with involvement of the central nervous system, followed by involvement of the circulatory system, pulmonary edema, and pulmonary hemorrhage, and this process happened very quickly [10]. When the central nervous system was in-

volved, patients could present with symptoms of vomiting, lethargy, limb weakness, gait disturbance, muscular convulsion, flaccid paralysis, etc, for which they should be placed in the intensive care unit (ICU). This period could last for days or weeks, and there is a possibility that the patients could self-improve, with a few of them left with sequelae. If the disease progresses into the stage of cardiopulmonary failure, the probability of death is about 80%; moderate or severe nervous system sequelae could occur in those who survive [19]. Therefore, early detection of the children with high risk of getting severe HFMD is the key to reduce mortality rate. This study showed that the risk factors include fever being the first symptom, the highest body temperature $\geq 38.5^\circ\text{C}$, herpes appearing at the isthmus of fauces, vomiting, convulsions, and lethargy. Consistent with the findings of the prospective clinical study conducted by Chang *et al.* [20] on HFMD from 2000–2006, the risk factors for nervous system involvement were duration of fever ≥ 3 days, the highest body temperature $\geq 38.5^\circ\text{C}$ and lethargy. Other studies found that the risk factors for occurrence of neurogenic pulmonary edema after nervous system involvement included hyperglycemia, elevated white cell count and acute flaccid paralysis [21]. Based on the EV71 positive finding of laboratory tests, clinicians can refer to the above findings to closely monitor children with a high risk of severe HFMD and deliver timely and effective treatment when necessary.

Grassroots level medical staff is the frontline to treat children with HFMD; it is a key issue for them to screen out the ones that have a high risk of severe HFMD. This study showed that the probability of developing into severe HFMD for children first diagnosed with HFMD or suspected cases is 0.38 times higher than that for those first not diagnosed as having HFMD. Therefore, timely diagnosis can reduce the risk of progressing to severe HFMD. At present, China's rural healthcare staff is

characterized by insufficient quantity, low quality, irrational structure, imbalanced allocation, etc. According to statistics, by the end of 2008, there were 2.21 healthcare technicians among every 1000 people, among which certified (assistant) physicians comprised only 0.94 [22]. Health administrative departments of various levels should strengthen the training and continued education for rural healthcare technicians, with a focus on investigation, prevention, surveillance, diagnosis, treatment of rural common diseases and handling of public health emergencies, to help rural doctors establish a thinking style of a general practitioner, so as to improve their ability to handle health issues commonly seen.

This study firstly introduced such relevant factors as patients' hospital visit behavior and the result showed that the risk factors for severe HFMD included self-medication before the hospital visit, taking antipyretics before the hospital visit, first visiting a village level clinic, and visiting a village doctor. The main result from the 4th National Health Service Survey (2008) revealed that around 36% of the Chinese rural population do not seek treatment after falling ill, among which 70% employ self-medication or purchase medication from a pharmacy for treatment [23]. Around 57% of rural residents choose to seek treatment at the village level clinics, and around 85% of these rural clinics are privately owned [23], characterized by small scale, outdated equipment, limited variety of medications, lack of professional knowledge, etc. Therefore, health education should be strengthened for rural residents to improve their awareness to seek treatment actively and early. Also, the focus of rural health industry should be rational allocation of health resources, optimization of health service systems and increased input into township and village level health institutions, to equip them to be able to handle major epidemics and public health emergencies.

The transmission route of EV71 is very complicated, and the prevention and control of EV71 is also a challenge due to the fact that there is no safe and effective vaccine or antiviral medications available. The most effective method to control the spread of EV71 is to monitor the activity of EV71, so as to set off an alarm at the early stage of the epidemic and avoid contact between the infected and the vulnerable. The surveillance system for EV71 includes clinical surveillance and lab surveillance, and it should be incorporated into the whole public health surveillance system, to detect the number of cases and clinical presentations of HFMD patients infected with EV71 timely, and to monitor the activity of EV71 and other neurotropic enteroviruses. Health administrative department could predict the occurrence of epidemics based

on the information provided by the surveillance system, so as to timely alarm the general population about the impending epidemic and effectively implement interventions. In order to respond to the spread of EV71 in the Asian-Pacific regions, Taiwan [24], Singapore [25], Malaysia [26] and other countries and regions have started surveillance on the activity of EV71. During the pandemic of EV71 in Taiwan in 1998, the local government closed schools and kindergartens to minimize contacts among children, and it is reported that these preventive measures significantly reduced the number of severe HFMD cases and mortalities [24]. The government of Singapore took similar intervention measures in children centers where there was a high density of children, complemented with education of the general population through the media, and the surveillance data revealed that the incidence of EV71 among children aged 0–4 years dropped significantly from 2001 to 2007 [25]. China could borrow from these experiences to establish a comprehensive surveillance system for EV71, to be able to control an epidemic of EV71 early, reduce the incidence and mortality of patients with nervous system related diseases, and the disease burden.

To sum up, during the epidemic of HFMD, the parent and clinician should attach great importance to the progress of children's diseases, if the local CDC detected that the type of virus causing the epidemic is EV71 and if the children are ≤3 years old, present with high fever, herpes appearing at the isthmus of fauces, lethargy, vomiting, convulsions, etc, to closely monitor the disease progression and timely and effectively treat the patients by referencing the lab test result. Health education should be strengthened for guardians and parents of children, to make sure that common knowledge is learned by them, so as to raise their awareness to actively seek treatment. Also, construction of grass root level medical facilities and training of clinical and laboratory staff should be reinforced so that the diagnostic and treatment capacity can be improved.

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