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Original Article

Risk factors of severe hand, foot and mouth disease in Shantou, China: a case-control study

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Abstract

Introduction: In clinical perspectives, how to distinguish a small proportion of children at risk of developing neurological complications from a large number of children with mild symptoms still remains a challenge for primary care doctors.

Methodology: From January 2012 to December 2015, 225 cases with severe hand, foot and mouth disease (HFMD) matched with 492 controls were enrolled in the age-matched, case-control study. Continuous variables were examined by univariate analysis using a chi-squared or Fisher's exact test, and categorical variables were reported by relative risks (odd's ratio). Multivariate logistic regression was used to analyze the independent risk factors for severe HFMD.

Results: Peak body temperature over 37.5℃, total duration of fever over 3 days, lethargy, enterovirus 71 (EV71) infection were independent risk factors for severe HFMD.

Conclusions: Peak body temperature over 37.5℃, total duration of fever over 3 days, lethargy, EV71 infection were independent risk factors for severe HFMD.

Key words: Hand, foot and mouth disease; children; risk factor; enteroviruses; diagnosis.

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Introduction

Hand, foot and mouth disease (HFMD) is a highly contagious disease with clinical features of fever, oral ulcers, and vesicular rashes on the hands, feet, and buttocks, which mainly affects children under 5 years of age [1]. It is commonly caused by enterovirus 71 (EV71), coxsackie virus A16 (CVA16) and coxsackie virus A6 (CVA6), and generally transmitted via the fecal-oral route, respiratory droplets, or close contact with infected individuals [2,3].

Data from Ministry of Health of the People's Republic of China showed that HFMD ranked first among the notifiable infectious diseases in 2010 and 2012, and the deaths caused by HFMD ranked fifth [4]. The largest Asia–Pacific HFMD epidemic broke out in Fuyang, China, in 2008 with approximately 490,000 infections and 126 deaths in infants and young children [5]. After the outbreak, a series of national guidelines (Ministry of Health of the People's Republic of China, 2008 and 2010 edition) for the prevention and management of HFMD have been implemented in China, leading to a declined rate of mortality ratio [6]. However, accumulating evidence indicates that mortality in severe cases is still increasing worldwide [7]. Shantou is an important coastal city in eastern Guangdong, China. It has a monsoon-influenced humid subtropical climate, which favors spread of the disease, especially in warmer months (from March to July).

Most HFMD cases are mild and self-limiting, however some cases, especially those related to EV71 infection, may deteriorate rapidly and cause severe complications in children, resulting in serious consequences, even sudden death [8]. The severe cases are often accompanied by neurological complications, including brain stem encephalitis, pulmonary oedema, and sudden cardiopulmonary collapse [9,10]. Therefore, prediction and early recognition of risk factors foreshadowing a severe case will enable physicians to focus special attention on it and provide timely intervention to avoid fulminant, intractable conditions. However, some clinical signs or indicators of severe HFMD are very subtle, particularly in young children, which may be overlooked in the first diagnosis, especially at the primary care setting, so how to distinguish a small proportion of children at risk of developing severe conditions from a large number of

children with mild symptoms still remains a challenge for primary care doctors.

Previous studies have shown that some clinical symptoms and laboratory findings are closely associated with occurrence of severe HFMD in children, such as duration of high temperature, lethargy, and lumbar puncture revealing pleocytosis [11-13]. However, it is still an open question as to which risk factors are critical in identifying symptoms suggestive of severe HFMD in terms of clinical decision-making. Hence we conducted the present study to evaluate the risk factors related to severe HFMD in children through a retrospective investigation of the demographic characteristics, clinical features and laboratory findings of the patients admitted to our hospital.

Methodology

Study subject and Grouping

This age-matched case-control study was conducted to assess the risk factors of severe HFMD by comparing the demographic characteristics, clinical features and laboratory findings between severe and mild HFMD children patients. The severity of disease was determined on the basis of Chinese guideline for HFMD diagnosis and treatment (Ministry of Health of the People's Republic of China, 2010 edition) [14]. The present study was approved by the Human Ethical Committee of Shantou University Medical College, China (No. SUMC-2016-69). According to the following definition, a total of 225 severe cases and 492 mild cases were randomly selected as the case group and the control group from a retrospective review of medical records diagnosed HFMD in our hospital between January 2012 and December 2015. Controls were matched in the ratio of 2:1 to cases for the year of presentation and age approximately. All cases were admitted to in-patient department, and controls were usually treated in out-patient department.

Definition of severe and mild cases

A severe case was considered to have more serious complications, such as nervous system involvement, respiratory and circulatory system disorders. The symptoms include poor spirits, drowsiness, delirium, headache, vomiting, limb tremor, myoclonus, nystagmus, ataxia, eye movement disorders, weakness, encephalitis, meningitis, acute flaccid paralysis (AFP), or cardiorespiratory failure.

A mild case was defined as having clinical onset of any of the following features, including maculopapular or vesicular rash on the palms and/or soles and vesicles or ulcers in the mouth.

EV71 detection

The EV71 detection method is based on a one-step RT-PCR assay of viral RNA using EV71-specific primers for the molecular identification of EV71. EV71-specific RT-PCR amplification is a rapid and simple identification method and requires lower installation and running costs. In the present study, a commercially available EV71 diagnostic kit (Da An Gene Co., Ltd, Guangzhou, China) was used to detect EV71 in stool specimens, and its sensitivity is 1×10^3 plaque-forming units/mL. A specimen is considered positive for EV71 if the amplification curve crosses the threshold line within 35.1 cycles.

Data collection and statistical analysis

Demographic characteristics, clinical symptoms and signs, and laboratory results were collected from the medical record database. We extracted the data of interest and investigated the explanatory variables in them to explore their association with the severity of HFMD. Data included demographic characteristics (gender, age, living area, attending kindergarten/school, guardian), hospital visiting behavior, clinical presentation (duration of fever, lethargy, vomiting, diarrhea, limb weakness), laboratory findings (total white blood cell count, neutrophil counts, lymphocyte counts), and etiological test (EV71). As the clinical data were collected retrospectively, the observation time was recorded according to the actual situation of the patients. The neurological signs and symptoms were recorded within 1 week after onset, and laboratory tests were done within 3 to 7 days after onset. The respiratory and circulatory data of severe cases were recorded in the most critical period within 72 hours.

The indicators were determined according to World Health Organization and national guidelines and our clinical experience. Peak body temperature ≥ 37.5℃ and total duration of fever ≥ 3 days were determined as clinical signs of severe cases. For laboratory diagnosis, total white blood cell count ≥ 10.8×10^9L, neutrophil count>65%, and lymphocyte count < 30% were considered abnormal biochemical presentation.

Data were analyzed by using STATA 12.0 software (StataCorp LP, College station, TX, USA). Continuous variables were expressed as mean ± SD. and compared by univariate analysis using a chi-squared or Fisher's exact test. Binary and categorical explanatory variables were examined by univariate conditional logistic regression and reported as relative risks (Odd's ratios) with 95% CI. A multivariate logistic regression was performed to examine the adjusted odd's ratios with

95%CI for risk factors significant in the univariate conditional logistic regression analyses. The variables were selected using a stepwise backward elimination approach using $p < 0.05$ for retention.

Results

From January 2012 to December 2015, 225 cases including 1 death and 492 controls without death were included in the analysis.

Table 1 shows the demographic characteristics and presenting history of all subjects. No significant difference was found between cases and controls regarding age and sex distribution, however severe cases were more likely to be male. Children living in rural area have significantly higher incidence of severe HFMD than those living in urban area (OR 1.68, 95% CI 1.20-2.37, p = 0.002). Children attending kindergartens or day-care centres have a higher risk of severe HFMD than those home-cared (OR 1.76, 95% CI 1.21-2.55, p = 0.002). Furthermore, patients first visited hospitals below county level have a higher risk to develop severe HFMD (OR 1.89, 95% CI 1.15-3.10, p = 0.007).

Table 2 presents the univariate analysis of clinical symptoms and laboratory findings of the subjects. Case subjects have significantly high proportion of symptoms including peaking body temperature over 37.5℃ (OR 2.86, 95% CI 2.01-4.08, $p < 0.001$), duration of fever more than 3 days (OR 3.25, 95% CI 2.30-4.59, $p < 0.001$), lethargy (OR 5.84, 95% CI 3.61-9.56, $p < 0.001$), vomiting (OR 9.23, 95% CI 5.79-14.91, $p < 0.001$) and limb trembling (OR 4.01, 95% CI 2.60-6.19, $p < 0.001$). Laboratory findings indicated the case subjects have significantly elevated ratios of abnormal counts of white blood cell (OR 3.40, 95% CI 2.39-4.84, $p < 0.001$), neutrophil counts (OR 11.75, 95% CI 7.94-17.41, $p < 0.001$) and lymphocyte counts (OR 4.91, 95% CI 3.44-7.01, $p < 0.001$). Etiological test shows the risk of EV71 infection in cases was significantly higher than in controls (OR 12.89, 95% CI 7.87-21.93, $p < 0.001$).

Table 3 presents the results of a multivariate logistical regression of possible risk factors of severe HFMD significant in the previous univariate conditional logistic analysis. The results showed that an increased risk of severity was significantly associated

Table 1. Univariate analysis of demographic characteristics and presenting history.

Demographic characteristics		Cases (n = 225)	Controls (n = 492)	OR (95% CI)	*P* value
Age, month (means ± SD.)		2.38 ± 1.48	2.57 ± 1.58	-	0.562
Gender	Female	73	189	Ref	
	Male	152	303	1.30 (0.92-1.84)	0.123
Living in rural area		147	260	1.68 (1.20-2.37)	0.002
Guardian	Parents	176	406	Ref	
	Grandparents	41	80	1.18 (0.76-1.82)	0.430
Attending kindergarten		71	102	1.76 (1.21-2.55)	0.002
Hospital level first visited	County or above	189	447	Ref	
	Below county	36	45	1.89 (1.15-3.10)	0.007

Table 2. Univariate analysis of clinical symptoms and laboratory diagnosis between cases and controls.

Risk factors	Cut point	Cases (n = 225)	Controls (n = 492)	OR (95%CI)	*P* value
Symptoms					
Peak body temperature	≥ 37.5°C	154	185	2.86 (2.01-4.08)	< 0.001
Duration of fever (days)	≥ 3	117	123	3.25 (2.30-4.59)	< 0.001
Lethargy		65	32	5.84 (3.61-9.56)	< 0.001
Vomiting		88	10	9.23 (5.79-14.91)	< 0.001
Diarrhea		3	6	1.08 (0.17-5.11)	0.91
Limb trembling		68	48	4.01 (2.60-6.19)	< 0.001
Laboratory findings					
Total WBC	> 10.8×10^9L	108	105	3.40 (2.39-4.84)	< 0.001
NEUT	> 65%	147	68	11.75 (7.94-17.41)	< 0.001
LYM	< 30%	155	153	4.91 (3.44-7.01)	< 0.001
EV71 infection		204	220	12.89 (7.87-21.93)	< 0.001

WBC, white cell count; NEUT, neutrophil count; LYM, lymphocyte count.

Table 3. Multivariate analysis on risk factors for severe HFMD.

Risk factors	OR (95%CI)	*P* value
Peak body temperature ≥ 37.5°C	1.98 (1.25-4.12)	< 0.001
Duration of fever ≥ 3 days	2.92 (1.59-5.77)	0.01
Lethargy	2.41 (1.34-5.12)	< 0.001
EV71 infection	5.25 (2.78-10.25)	< 0.001

with the presence of peaking body temperature over 37.5℃ (OR 1.98, 95% CI 1.25-4.12, p < 0.001), high fever for more than 3 days (OR 2.92, 95% CI 1.59-5.77, p = 0.01), lethargy (OR 2.41, 95% CI 1.34-5.12, p < 0.001), and EV71 infection (OR 5.25, 95% CI 2.78-10.25, p < 0.001).

Discussion

Comprehensive understanding of clinical symptoms, laboratory tests and demographic features of HFMD patients is crucial to provide timely treatment to reduce the incidence of severe HFMD. The three categories of features may help doctors predict a patient's possibility to develop into severe HFMD at an early stage. In the present study, we identified several risk factors associated with severe HFMD in clinical features, laboratory test results, and demographic characteristics. Our findings indicate that the presence of peak temperature over 37.5℃ for more than 3 days, lethargy, a raised total WBC count, and EV71 positivity may more likely relate to severe HFMD. Furthermore, clinicians should pay more attention to children who live in rural area and ever visited hospitals below county level.

Many studies have confirmed the association between one or more above-mentioned clinical symptoms and severe HFMD, which often companied by CNS manifestation [6,9,15]. For example, EV71 associated severe HFMD is usually characterized by brainstem encephalitis, a distinctive form of encephalitis with stereotypic neuropathological feature.

In the development of severe HFMD, body temperature over 37.5℃ or higher, duration of fever longer than three days, lethargy, vomiting and limb weakness are generally considered as indicators of CNS involvement [15,16]. As the onset of neurological involvement to fulminant cardiorespiratory failure would be very rapid, once obvious clinical manifestations are found, the optimal timing of intervention has been missed. Therefore, it is imperative for a clinician to predict or recognize subtle indicator before the onset of neurological involvement particularly in younger children. Interpreting risk factors related to the severity of HFMD could help doctors decide whether to admit children HFMD patients into hospital for close monitoring or to alert their parents or care-givers to keep a close watch after leaving out-patient.

Our findings on clinical symptoms are consistent with several previous studies [15,17,18]. Ooi *et al*. identified total duration of fever over 3 days, peak temperature over 38.5℃ and history of lethargy as independent risk factors for neurological involvement, which was evident by cerebrospinal fluid (CSF) pleocytosis and indicating severe HFMD progression. They also pointed out that the three readily elicited clinical risk factors could help clinicians to decide whether patients should be hospitalized for further monitoring and treatment [15]. Recently, a meta-analysis including 19 studies pointed out that duration of fever over 3 days, body temperature over 37.5℃, lethargy and vomiting significantly increased the risk of severe HFMD [11]. A two-phase prospective clinical study identified and validated that total duration of fever over 3 days, peak temperature over 37.5℃ and history of lethargy was independent risk factors for neurological involvement which was verified by CSF pinocytosis. Chang *et al.* found a history of lethargy may be a useful clinical sign for CNS involvement during the first 2 days of febrile illness, which is typically undifferentiated and subtle, even to the experienced clinician [16]. Yang *et al.* thought leg trembling was a significant risk factor of severe cases, which is more relevant to CNS involvement after EV71 infection [19].

Viral test and blood routine examination can help clinicians to verify their judgment, as the findings can alert them keep close monitoring to prevent the onset of serious neurological complications. In the present study, viral etiological test confirmed that EV71 infection was strongly related to the development of severe HFMD. HFMD and herpangina are caused by co-circulating enteroviruses, including CA (serotypes 2-8, 10, 12, 14, 16) and EV71, which is a benign, self-limiting illness in most cases. However, the epidemics in Asia has shown the infection caused by EV71 can result in severe, even fatal, systemic complications in children, particularly those under five years of age [6,8,20,21]. Many previous studies revealed that the positivity rate of EV71 was significantly higher in

severe cases of HFMD [15,17,19,22,23]. However, virus isolation and identification are often laborious and time-consuming, which is not convenient for clinical decision-making in out-patient department, so careful observation of clinical symptoms and reading of biochemical examination become particularly crucial for primary care doctors [3].

Our laboratory findings showed a raised white cell count with raised neutrophil counts and decreased lymphocyte count were more likely seen in severe cases, which is in line with biochemical findings in other studies [18,19]. Laboratory abnormalities are commonly observed in children at early stage of severe disease, which include a raised white cell count with relative neutrophilia, hyperglycaemia and elevated CSF lactate [16,24,25]. Recently, a systematic review including 37 studies, mostly from Mainland China and Taiwan, indicated the prevalence of WBC counts and leukocytosis increased with the severity of the illness [26]. Although these indicators seem more likely to present in severe HFMD cases, their diagnostic value is limited for clinically predicting children at high risk of severe HFMD, particularly in primary care settings, as leukocytosis and hyperglycemia appear later in the course of fulminant cardiorespiratory failure in our opinion. Furthermore, CSF pleocytosis, an objective indicator for CNS involvement, could be applied to predict the development of an early course of severe HFMD within the first 2 days of febrile illness [15]. Standalone risk factors revealed by univariate conditional logistic regression may provide us a guide to read symptoms and laboratory results, however they are very likely subjected to many confounders. Following multivariate logistic analysis showed that peak body temperature over 37.5°C, total duration of fever over 3 days, history of lethargy and EV71 infection remained as independent risk factors of severe HFMD, which are thought readily elicited in clinical practice [15].

We previously reported the sociodemographic and behavioral risk factors related to severe HFMD in a multicenter, prospective case-control study. The study revealed that the children of migrant workers and children attending kindergartens were significantly associated with a severe outcome of HFMD [23]. In the present study, we found children attending kindergarten have higher possibility being affected by severe HFMD than their peers.We speculate that the children attending kindergartens have more frequent and close contact with EV71-infected children than home-cared ones. The finding is in line with several previous reports indicated kindergarten attendance and prior exposure to HFMD cases as the risk factors for severe HFMD or EV71 infection [16,23]. We also found those children living in rural area and first visited hospitals below county level have higher odds to develop severe HFMD. The results may be linked to their parent's hospital seeking behavior, because the rural parents often lack knowledge of the disease due to low education and inferior economic condition, which underlines an imperative necessity of health education in rural areas [27].

The strength of our study was a thorough investigation of many aspects relating to the severity of HFMD, including clinical symptoms, laboratory parameters, demographic characteristics and hospital visiting behavior. The data could be valuable to clinical diagnosis and further research for pediatric clinicians. However, it is noteworthy that the present study has several limitations. First, it is a retrospective one which is subject to potential selection biases. Second, our study was hospital-based, and the sample size was relatively small compared to epidemiological investigations, so its conclusion requires confirmation by further in-depth observations and clinical trials.

Conclusions

In conclusion, clinicians should be cautious of the presence of a high fever over 37.5°C, duration of fever over 3 days, lethargy, and EV71 infection. The risk factors provide a useful guide for clinicians to decide the need to hospitalize as well as to monitor disease progression in children with HFMD.

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