Original Article

Investigation on Factors Associated with Severe Hand, Foot and Mouth Disease

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Objective To analyze the clinical and laboratory features of patients with mild and severe HFMD to identify early predictive or diagnostic markers for severe cases.

Methods Samples of feces, nasopharyngeal-swab specimens, peripheral blood, serum and cerebral spinal fluid were collected. Postmortem pathological examination was conducted on 2 dead patients with complication due to neurogenic pulmonary edema. Reverse transcription-polymerase chain-reaction (RT-PCR), culture and isolation of enterovirus 71 (EV71) were performed to detect EV71 infection. Both univariate and multivariate logistic analysis were used to identify factors associated with severe cases.

Results EV71 was mainly responsible for HFMD. In this study, 5 isolated EV71 strains belonged to C4 gene subtype. Compared with mild patients, EV71-RNA detection rate was higher and CoxA16 detection rate was lower among severe patients ($P < 0.01$). Inflammatory cell infiltration in the lung, cardiac and liver tissues were mild by postmortem pathological examination. It was found that body temperature, vomiting, limb tremor, neutrophil, blood glucose and EV71 infection were significantly related to the severe cases by univariate logistic analysis. However, after multivariate logistic regression analysis, only vomiting (OR 16.1, CI 2.3-110.5, $P < 0.01$) and limb tremor (OR 117.6, CI 13.8-1004.5, $P < 0.01$) were significantly and independently correlated with the severe cases.

Conclusions EV71 was mainly responsible for HFMD, particularly for severe cases. Vomiting and limb tremor were predictive markers for severe cases.

Key words: Hand, foot and mouth disease; Enterovirus 71; Predictive marker; Severe cases

Enterovirus 71 is emerging as a major cause to HFMD, which mainly attacks children, especially those who under 4 years old, however other enteroviruses such as Coxsackievirus A16 (CoxA16) and Echovirus are also responsible for HFMD.¹ HFMD is characterized by fever, hand, foot and mouth rashes and vesicles, mouth ulcer and herpangina, with a small proportion (about 10% HFMD) of patients complicated with central nervous system diseases including aseptic meningitis, brainstem encephalitis, neurogenic pulmonary edema or hemorrhage, acute flaccid paralysis and circulation failure.^{2,3} Patients died of acute onset of neurogenic

pulmonary edema (PE) and/or hemorrhage with rapid progression of cardiopulmonary failure within hours after admission.^{3,4} The pathogenesis of PE is still unknown. Chang et al postulated that viral destruction of CNS may be the cause of death.⁵ Central sympathetic activation results in systemic vasoconstriction and the shift of blood volume to the pulmonary region of the circulatory system.^{6,7} Predicting which patients would potentially progress into severe or critical cases associated with HFMD remains unclear. In fact, no clinical feature was identified to predict which patients were likely to develop severe disease resulting in death.⁹ However, it's very urgent for clinicians to identify predictive factors as early as possible.

In 2008, HFMD broke out in Shenzhen City, a total of 124 mild cases and 60 severe cases were enrolled into this study from May to July, 2008. Seven cases experienced brainstem encephalitis and 2 patients

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died of neurogenic pulmonary edema and brainstem encephalitis. The clinical and laboratory data of both mild and severe patients were collected and analyzed to identify the predictive factors of severe cases and help clinicians make diagnosis and treatment punctually and properly, and finally decrease mortality and disability rates of severe cases.

MATERIALS AND METHODS

Case definition

Symptoms characterized by oral ulcers plus skin vesicles or ulcers spreading over the gingival and buccal or hard palate were designed as defined HFMD, even in the absence of any skin manifestations.³ Herpangina was defined as oral ulceration on anterior tonsillar pillars, soft palate or buccal mucosa, or uvula HFMD patients had mouth ulcers plus vesicular rash on the hands, feet, knees, or buttocks.⁵ EV71 infection was defined as the isolation of the virus, amplification of the virus RNA or test of specific IgM positive for EV71 from at least one site of the body (nasal and throat swabs, stool and cerebral spinal fluid), or the serum IgG at convalescent stage is 4 folds or more than that at acute phase, or convalescent serum EV71 IgG becomes positive at acute phase. Autonomic nervous system (ANS) dysregulation was defined by the presence of cold sweating, mottled skin, tachycardia, tachypnea and hypertension. PE was defined as respiratory distress with tachycardia, tachypnea, rales, and pink frothy sputum that developed after ANS dysregulation, together with chest radiograph that showed bilateral pulmonary infiltrates without cardiomegaly.³ Brainstem encephalitis (BE) was characterized by myoclonus, ataxia, nystagmus, oculomotor palsies, and bulbar palsy in various combinations, with or without neuroimaging. Symptoms of patients with severe HFMD were composed of high fever ($> 38^{\circ}\text{C}$), vomiting, tachypnea, and indications of neurological complications (aseptic meningitis, encephalitis, or acute flaccid paralysis) or cardiopulmonary complications (pulmonary edema, pulmonary hemorrhage or myocarditis). Patients with one of these complications were considered as severe cases.³

Study population

From May to July, 2008, a total of 184 patients with HFMD (including 124 mild cases and 60 severe cases) were recruited into this study after informed written

consent was obtained. All patients were divided into 2 groups (mild and severe cases) according to the diagnostic criteria for HFMD published in 2008 by the Ministry of Health of China. Of these, 110 were defined as EV71 infection by isolation of EV71 or amplification of EV71 gene by RT-PCR.

Sample collection

Throat swabs, stool and vesicle specimens were collected daily for 7-15 days after patients were admitted to the hospital (until amplification of EV71 gene by RT-PCR was undetectable), serum and peripheral heparinized blood from all the patients and CSF (cerebral spinal fluid) from severe cases was collected as well. For two severe cases died of NPE, autopsy of lung, liver and heart tissues was conducted to make a pathological diagnosis.

Routine blood tests and biochemistry detections

Routine blood, routine CSF, and liver and cardiac biochemistry detections were conducted according to the standardized procedures.

Viral isolation and culture

Nasopharyngeal swab specimens were collected and kept in viral transport medium and subsequently inoculated monolayers of Vero, rhabdomyosarcoma (RD cells) and MRC-5 cells for viral isolation within 24 h. The cultures were incubated at 37°C and monitored daily for 14 days for viral cytopathic effect (CPE). If typical CPEs were observed, immunofluorescence staining for identification of EV71 was performed with both monoclonal antibodies to EV71 (Mabs 3323 and 3324; Chemical International). Mab 3323 can cross-react with both Cocksackievirus A16 and EV71, however, Mab 3324 only specifically reacts with EV71.⁵ EV71 infection was defined by response of viral supernatant to both of the Mabs. Identification of EV71 was further confirmed by neutralizing tests with polyclonal antibodies against EV71, which were produced in rabbit serum.

RT-PCR and full length sequence analysis

Clinical specimens including plasma, CSF (cerebrospinal fluid), rectal and throat swabs were used for viral RNA extraction according to the conventional protocols using Trizol Reagent (Sigma Chemical Co.). Reverse transcription and quantitative real-time PCR was carried out for detection of

EV71 gene with a ABI 7700 PCR machine (Applied Biosystems) by TaqManTM technology. The specific primers for EV71 published by Chinese Ministry of Health were used to detect EV71 infection. The forward primer: 5'-CCCCTGAATGCGGCTAATC-3', 5'-non-coding conserved region); the reverse primer: 5'-TGGCCAATCCAATAGCTATATGG-3', 5'-non-coding conserved region). RNA of the 5 EV71 strains was extracted and the full length gene sequences were examined. Alignment of the full length EV71 gene sequence was undertaken using the Clustal method of DNASTAR MegAlign. A phylogenetic tree was constructed by the neighbor-joining method with MEGA 3 (Molecular Evolutionary Genetics Analysis, Version 3.0). The reliability of the neighbor-joining tree was estimated by bootstrap analysis with 1 000 pseudo-replicates.

Postmortem pathology

Two HFMD patients complicated with pulmonary edema and brainstem encephalitis died and postmortem pathological examination were performed. Pulmonary, liver and cardiac tissues were collected through puncture operation, and a routine pathological staining was taken according to the conventional manufacture's instruction.

Statistical analysis

The differences between the two groups in numeric, categorical, and ordinal scales were analyzed using Student's *t* tests or Fisher's exact tests, a χ^2 test with Yates's correction. Both univariate and multiple logistic regression analysis were used to examine the multivariate-adjusted odds ratios for risk factors associated with severe cases. A *P* value of less than 0.05 indicates a significant difference in statistics.

RESULTS

Analyses of clinical manifestations, complications and laboratory profiles of HFMD

Among the 60 severe cases, males accounted for 78.3% (47/60) and females accounted for 21.7% (13/60). Mean age in severe cases was 33.66 ± 46.93 months, with a range from 37 days to 15 years. The clinical and laboratory data were listed in Table 1 and Table 3. The severe cases had fevers with higher temperature, more shortness of breath, severer mouth ulcers and limb tremors than the mild cases (*P* < 0.05) as shown in Table 1. Moreover, The WBC

(white blood cell counts) and blood glucose levels of the severe cases were significantly higher compared with those of the mild ones (Table 3). In addition, shortness of breath, vomiting, extremity tremor and extremity convulsion occurred more frequently in the severe cases than it did in the mild ones. The severe patients were complicated with aseptic meningitis, brain-stem encephalitis, NPE, myocarditis, heart failure or acute flaccid paralysis (Table 2). Lumbar puncture was performed in a severe patients and the routine biochemistry detections for CSF were carried out. The CSF profile was similar to viral meningitis except for a slight increase in WBC count (data were not shown).

Isolation and culture of EV71

The stool samples were kept in the transport medium and cultured in the rhabdomyosarcoma cell line RD for 5 days. Ten EV71 strains in the stool were isolated from the 20 cases with EV71 infection. Fluorescent monoclonal antibodies to EV71 and CoxA16 viruses were used to detect EV71 infection. Yellow cells indicated EV71 infected RD cells (Figure 1).

Detection of EV71 genes from different body sites

EV71 and CoxA16 genes were amplified from feces, throat swab, CSF and peripheral blood of the patients by RT-PCR and the positive rates of detection were analyzed. Nine EV71 strains were isolated and identified from 20 patients with positive amplification of EV71 genes by RT-PCR assay. It was found that EV71 gene detection rate from stool in severe patients was significantly higher than that in mild patients (76.7% vs 35.5%, *P* < 0.01). Moreover, EV71 gene detection rate from feces was significantly higher than that from throat swab, and no EV71 gene was found in CSF by RT-PCR assay (Table 4).

CoxA16 was a major pathogen for HFMD in children worldwide between 1980s and 1990s. Genes of CoxA16 from patients' stool, throat swab or CSF were amplified by RT-PCR assay. It was demonstrated that CoxA16 infection contributed to a small proportion of cases, particularly less common in severe cases. But EV71 infection was mainly responsible for mild cases, and were more frequent in severe cases and 2 cases dying of NPE and brainstem encephalitis (Table 5). These findings demonstrated that EV71 infection contributed to the majority of mild cases and most severe cases.

Table 1. Clinical features of the mild and severe patients [n(%)]

Symptoms	Mild (n = 121)	Severe (n = 60)	χ^2 (Chi Square/Fisher's exact tests)	P values
Fever	108 (87.1)	59 (98.3)	4.8	0.03
Rash	124 (100.0)	58 (96.7)	1.7	0.2
Mouth ulcer	31 (25.0)	42 (70)	34.2	< 0.001
Cough	39 (31.4)	12 (20)	2.6	0.1
Dyspnea	0	40 (66.7)	98.4	< 0.001
Vomiting	14 (11.3)	45 (75)	66.7	< 0.001
Diarrhea	1 (0.8)	4 (6.7)	3.3	0.07
Extremity tremor	6 (4.8)	56 (93.3)	137.8	< 0.001
Extremity convulsion	0	8 (13.3)	16.4	< 0.001

Table 2. Complications of the severe cases [n(%)]

Complications	Cases
Aseptic meningitis/encephalitis	60 (100)
Brainstem encephalitis	9 (15)
NPE	6 (10)
Pulmonary hemorrhage	2 (3.3)
Myocarditis	16 (26.7)
Heart failure	6 (10)
Acute flaccid paralysis	13 (21.7)
Myelitis	6 (10)
Acute stress ulcer	3 (5)
Respiratory failure	3 (5)
Elevated blood glucose	7 (11.7)
Electrolyte disorder	1 (1.7)
Shock	4 (6.7)

Table 3. Analysis of laboratory findings of the mild and severe patients

Variables	Mild (n = 124)			Severe (n = 60)		
	Max	Min	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD
Age (months)	144	7	31.6 $\pm$ 20.2	180	1	33.7 $\pm$ 46.9
Body temperature (°C)	40.7	36.3	38.4 $\pm$ 0.9	40.7	36.8	39.2 $\pm$ 0.6
WBC ($\times 10^9/L$)	29.99	2.5	9.9 $\pm$ 4.2	35.1	5.7	10.5 $\pm$ 4.3
Neutrophil (%)	82.2	16.3	52.0 $\pm$ 14.2	93.2	23.2	50.1 $\pm$ 14.9
GLU (mmol/L)	17.66	2.1	5.2 $\pm$ 1.9	26.7	3.1	6.4 $\pm$ 3.1
CRP (mg/L)	62	< 1	6.5 $\pm$ 7.87	21.0	< 1	4.3 $\pm$ 6.1
AST (U/L)	150	23	41.9 $\pm$ 20.3	145	33	29.2 $\pm$ 50.8
CK (U/L)	786	2	131.8 $\pm$ 120.3	1810	55.0	120.6 $\pm$ 222.8
CK-MB (U/L)	302	0.2	29.6 $\pm$ 30.9	151.0	17.0	13.8 $\pm$ 19.3

Notes: Max represents maximal value, Min represents minimal value. GLU represents glucose; CRP represents C reaction protein; AST represents aspartate aminotransferase; CK represents creatine kinase; CK-MB represents creatinine kinase isoenzyme MB.

Table 4. Amplification of EV71 RNA in patients' feces by RT-PCR [n(%)]

Groups	Positive	Negative
Mild cases	25 (10.2)	99 (89.8)
Severe cases	28 (46.7)	32 (53.4)

Notes: EV71 gene detection rate in the feces of the severe cases was significantly higher than that in the mild cases, $\chi^2 = 13.9$, $P < 0.001$.

Table 5. Detection of CoxA16 virus genes in patients' feces by RT-PCR [n(%)]

Groups	Positive	Negative
Mild cases	27 (21.8)	97 (78.2)
Severe cases	5 (8.3)	55 (91.7)

Notes: CoxA16 virus gene detection rate in feces of the mild cases was significantly higher than that in the severe cases, $\chi^2 = 5.08$, $P < 0.05$.

Table 6. Univariate logistic analysis of clinical and laboratory parameters of the mild and severe cases

Variables	β	Standard errors	Wald	P	OR	95% CI	
						Upper confidence limits	Lower confidence limits
Age	0.00	0.00	0.16	0.686	1.00	1.81	105.05
Fever	2.62	1.04	6.41	0.011	13.78	0.01	0.09
Body temperature	1.28	0.27	22.14	0.000	3.60	9.77	50.54
Oral ulcer	-3.58	0.57	38.96	0.000	0.03	32.75	287.89
Cough	-0.66	0.38	3.04	0.081	0.51	1.12	1.25
Vomiting	3.10	0.42	54.74	0.000	22.22	1.07	1.65
Diarrhea	1.81	1.17	2.41	0.121	6.11	1.00	1.00
Limb tremor	4.58	0.55	68.08	0.000	97.09	3.70	16.08
WBC	0.03	0.04	0.58	0.447	1.03	0.99	1.01
Neutrophil	0.17	0.03	35.04	0.000	1.18	2.11	6.15
CRP	-0.02	0.03	0.30	0.585	0.98	0.24	1.09
Glucose	0.28	0.11	6.42	0.011	1.33	0.62	60.09
AST	-0.05	0.02	11.06	0.001	0.95	0.96	1.11
CK	0.00	0.00	0.20	0.655	1.00	0.93	1.04
CK-MB	-0.12	0.02	23.73	0.000	0.89	0.92	0.98
EV71	2.04	0.37	29.70	0.000	7.71	0.85	0.93
CA16	-0.88	0.53	2.81	0.093	0.41	0.15	1.16

Notes: OR represents odds ratio.

Table 7. Multiple logistic analysis of clinical and laboratory parameters of the mild and severe cases after univariate logistic analysis

Variables	β	Standard errors	Wald	P	OR	95% CI	
						Upper confidence limits	Lower confidence limits
Body temperature	1.831	0.968	3.575	0.059	6.238	0.935	41.617
Oral ulcer	-4.799	1.322	13.175	0.000	0.008	0.001	0.110
Vomiting	2.777	0.984	7.969	0.005	16.069	2.337	110.487
Limb jitter	4.768	1.094	18.986	0.000	117.645	13.779	1 004.479
Blood glucose	0.273	0.184	2.203	0.138	1.313	0.916	1.882
CK-MB	-0.069	0.021	10.616	0.001	0.933	0.895	0.973
Constant	-71.762	37.528	3.657	0.056	0.000		

Notes: OR represents odds ratio.

Figure 1. Identification of EV71 infection using fluorescent monoclonal antibodies against EV71 or CoxA16 virus (immunofluorescence staining).

Notes: Normal controls of the rhabdomyosarcoma cell line RD were observed by light microscopy (1A). Cytopathic effects on the RD cells (1B). Yellow fluorescent indicates EV71 infected the RD cells (magnification $100\times$ in Figure 1C and magnification $200\times$ in Figure 1D).

Figure 2. Daily and cumulative numbers of cases with EV71 conversion to undetectable level from positivity in the stool during the 15 days after disease onset.

Notes: Red bars indicates the number of cases with EV71 infection daily, and the curve indicates the cumulative cases of confirmed EV71 infection during the period of 15 days after symptoms onset.

Figure 3. Gene subtype and phylogenetic tree of VP1 of EV71 strains.

Notes: Underlines represented 5 isolated EV71 strains, which belong to C4 gene subtype. One (SZ/08-121/08/CHN) of them came from a severe patient, another (SZ/08-605/08/CHN) from a critically ill case dying of NPE, and the other 3 ones (SZ/08-01/08/CHN, SZ/08-04/08/CHN, SZ/08-28/08/CHN) from 3 mild cases.

Figure 4. Postmortem histopathology from 2 cases dying of NPE (HE staining).

Notes: Lung tissue sections (A, amplification 100 ×), liver sections (B, amplification 100 ×) and cardiac sections by H&E staining (C and D, amplification 100 ×).

Dynamic viral shedding

Dynamic detection of EV71 infection was once determined daily by RT-PCR assay until it become undetectable. The feces of 34 mild cases was detected consecutively by RT-PCR assay since they were admitted to hospital. It was found that EV71 genes in the stool of 34 mild cases couldn't be detected by RT-PCR assay 15 days after disease onset. On the 8th day after disease onset, EV7 genes of 24 patients (70.6%) became undetectable by RT-PCR assay (Figure 2). This indicated that the viral loads of the majority of mild cases were undetectable on the 8th day after onset and all viruses of mild cases were cleared on the 15th day after the onset of disease.

Gene subtype and phylogenetic tree of VP1 of EV71 strains

The 5 full-length genes of EV71 were sequenced and the whole genes of EV71 was analyzed and compared with different strains across the world using the Clustal method of DNASTAR MegAlign. One (SZ/08-605/08/CHN) of 5 strains came from a girl, who presented with fever and hand, foot rashes for 2 days, shortness of breath for 7 h on admission and died of NPE and brainstem encephalitis within a few hours after admission. Another strain came from a severe case (SZ/08-121/08/CHN), the remained 3 strains were from mild cases. Genetic phylogenetic analysis indicated EV71 VP1 gene spreading in Shenzhen City belonged to C4 and was the closest to Fuyang of Anhui strain (EV71/Fuyang.Anhui.P.R.C/17.08/2/08/CHN) (Figure 3), with 98% identity with the Fuyang EU703812 strain; 99% identity with the strain of SHZH03 of 2003 Shenzhen epidemic strain. No significant differences in nucleotide or amino acid homologies among 2008 Shenzhen EV71 strains and strains from other parts of China were found. The nucleotide and amino acid sequences of VP1 of 2 severe strains and the 3 mild strains were compared, and no virulent genes related to neurogenic pulmonary edema were identified.

Pathology

A 4-year-old girl and a 2-year-old boy died of neurogenic pulmonary edema, and postmortem pathological examinations were conducted. Light microscopic observation showed partial alveolar space was infiltrated by fibrinousexudates, erythrocytes, macrophages and neutrophils, with broadening alveolar septum and expanding vessels congested

with neutrophils infiltration, which was in agreement with interstitial pneumonia (Hematoxylin and Eosin staining H&E, original magnification 200 ×) (Figure 4A). Liver sections showed hepatocytes vacuolar degeneration and edema with portal areas infiltrated with a few lymphocytes (Figure 4B). Sections of cardiac tissue showed myocardial cell vacuolar degeneration and interstitial edema without apparent inflammatory cells infiltration (Figures 4C and 4D).

Univariate and multiple logistic regression analysis

Multiple factors were correlated with severe cases based upon univariate logistic regression analysis, which included body temperature, vomiting, limb tremor, peripheral blood neutrophil cell count, blood glucose level and EV71 infection, with mean odds ratio ranging from 1.33 to 97.09. However, after multivariate logistic analysis, it was found that only vomiting and limb tremor were correlated with severe cases, and limb tremor was highly independent related to severe cases (Tables 6, 7).

DISCUSSION

In 2008, HFMD outbreak mainly hit Southern and Eastern China, particularly Fuyang City of Anhui Province, Guangdong Province, Shandong Province, Shanghai and so on. Shenzhen was also seriously attacked by the epidemic. In this study, it indicated that human enterovirus 71 (HEV71) was the most common virus responsible for HFMD, which contributed to the majority of severe cases and both of the 2 fatalities. CoxA16 only accounted for a small proportion of HFMD. It demonstrated that EV71 is a predominant killer of HFMD patients in Shenzhen area, especially for severe patients. However, CoxA16 often results in mild cases, seldom for severe cases complicated with NPE and brainstem encephalitis.^{10,11} It also showed EV71 epidemic strain spreading in Shenzhen area belonged to sub-genotype C4. After the whole gene sequences of 5 EV71 strains were blasted and analyzed, no probable virulent gene contributing to neurogenic pulmonary edema, brainstem encephalitis was found.

From the pulmonary, liver and cardiac pathological results upon routine H&E staining, it showed that the inflammatory responses in lung, cardiac and liver tissues of both dead children were mild, and characterized by interstitial edema, tissue cell degeneration, rare cell necrosis, in agreement

with previous reports.¹² Postmortem pathological examination showed extensive damage to the medulla and pons.¹³ This was quite different from pulmonary pathology of acute respiratory distress syndrome (ARDS) caused by other viruses such as SARS (severe acute respiratory syndrome) Coronavirus, human avian influenza virus.^{14,15} It also indicated pulmonary edema may originate from the damage of central nervous system, while not resulted directly from pulmonary EV71 infection.

Our study showed multiple factors including body temperature, vomiting, limb tremor, peripheral blood neutrophil cell count, blood glucose level and EV71 infection were correlated with severe cases based upon univariate logistic analysis. However, after multivariate logistic analysis, only vomiting and limb tremor were independently associated with severe cases, especially limb tremor was highly independent related to severe cases. Previous reports indicated that hyperglycemia, leukocytosis, and loss of limb strength were significant risk factors for NEP, and hyperglycemia was the most significant prognostic factor after multiple logistic regression analysis.⁵ Another study showed that myoclonus with sleep disturbance was the most important early sign of EV71 infection with CNS (central nervous system) involvement,¹⁶ which was similar to our study. Severe cases were complicated with central nervous system diseases. Limb tremor usually represents damage or inflammation of CNS, vomiting also indicates higher pressure of intracranial hypertension or CNS disorders. Higher blood glucose level represents endocrine system disorders. Moreover, CNS can regulate blood glucose level through pituitary-hypothalamus-adrenal gland axis.^{17,18} Several hormones such as corticoid, adrenaline, glucagon can affect blood glucose content as well. Hyperglycemia can be mediated by the action of adrenaline or glucagon on the liver, and by direct innervation of the liver.¹⁹ The hypothalamus can also modulate adrenal gland to secrete more corticoid because of CNS disorders. In this study, it was not found that hyperglycemia was an independent risk factor for severe cases, but this may be due to the small number of cases (only 6) complicated with NPE.

In conclusion, it showed EV71 infection was a major cause and killer for patients with HFMD in 2008 Shenzhen HFMD epidemic outbreak. Through multiple logistic regression analysis, it showed vomiting and limb tremor were related independently

to severe cases. These indicated vomiting and limb tremor can probably be taken as useful markers for early prediction of severe patients.

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ABBREVIATIONS

RT-PCR, reverse-transcription polymerase-chain-reaction; EV71 enterovirus, HFMD hand-foot-mouth disease; CPE, cytopathic effect; CSF, cerebrospinal fluid, NPE, neurogenic pulmonary edema.

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