RISK FACTORS FOR SEVERE HAND, FOOT AND MOUTH DISEASE

Somchai Owatanapanich[1], Rochana Wutthanarungsan[1], Wipaporn Jaksupa[1] and Usa Thisyakorn[2]

[1]King Narai Hospital, Lopburi; [2]Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract. We studied risk factors associated with severe hand, foot and mouth disease (HFMD) caused by enteroviruses among patients aged less than 15 years admitted to King Narai Hospital, Lopburi, Thailand during 2011-2013. Cases were divided into either mild or severe. Severe cases were those with encephalitis, meningitis, myocarditis, pneumonia, pulmonary edema or respiratory failure. Risk factors for severe infection were evaluated using univariate and multivariate logistic regression analysis. One hundred eighteen patients met the case definition of HFMD. Of these, 95 (80.5%) were classified as mild cases, and 23 (19.5%) as severe cases; there were 5 deaths (4.2%). Of the 23 severe cases, 9 were infected with coxsackievirus A16 (CA16), 8 with enterovirus 71 (EV71) and 4 with both EV71 and CA16. The most common presentations among the severe cases were: seizures (74%), pneumonia (39%), encephalitis (39%), and meningitis (13%). The clinical manifestations significantly related to severe HFMD on univariate analysis were highest body temperature ≥39.0ºC, duration of fever ≥3 days, absence of skin lesions, diarrhea, dyspnea, seizures and hyperglycemia. The clinical manifestations significantly related to severe HFMD on both univariate and multivariate analyses were age less than 1 year, absence of oral lesions and drowsiness/lethargy. Clinicians should be aware of these factors. Early recognition of severe cases is important to increase the rates of successful outcomes and reduce mortality.

Keywords: hand, foot and mouth disease, severe, risk factor

INTRODUCTION

Over the past decade, several outbreaks of hand, foot and mouth disease (HFMD) have been reported from countries in the Western Pacific Region, including China, Japan, Taiwan, Malaysia, Vietnam and Singapore (WHO, 2011). The disease is causing an increasing threat to public health worldwide (WHO, 2011). HFMD usually affects young children aged <5 years. The common manifestations of HFMD are fever and vesicular exanthema on the palms, soles and mouth. Most infections are mild and self-limited. Severe cases may be complicated by interstitial pneumonitis, meningitis, encephalitis, pulmonary edema, and myocarditis (Chang *et al*, 1999; Shah *et al*, 2003; Shekhar *et al*, 2005). Factors associated with severe or fatal HFMD include young age, absence of mouth ulcers, vomiting, tachycardia,

Correspondence: Professor Usa Thisyakorn, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Road, Pathum Wan, Bangkok 10330, Thailand.
Tel: 66 (0) 2354 7584; Fax: 66 (0) 2354 7584
E-mail: usa.thi@mahidol.ac.th

lethargy, hyperglycemia, and leukocytosis (Chong *et al*, 2003; Li *et al*, 2012; Fang *et al*, 2014). However, no reliable predictors of severe disease have been identified. Early recognition of severe cases and timely intervention are crucial to prevent cardiorespiratory failure, increase the ratio of successful outcomes and reduce mortality (Pan *et al*, 2012).

The major causes of HFMD are EV71 and CA16; both of which are non-enveloped, single-stranded RNA viruses belonging to family Picornaviridae (Xu *et al*, 2012). Distinguishing between pathogens clinically is difficult. Some reports have suggested that despite the close genetic relationship between EV71 and CA16, only EV71 has the potential to cause neurologic disease in acute infection and may result in a fatal outcome (Alexander *et al*, 1994; Chan *et al* 2000).

Data regarding the epidemiology and clinical features of severe HFMD in Thailand are limited. Factors associated with severe disease are unclear. Since 2001, HFMD has been listed as a notifiable disease in Thailand and herpangina (HA) was added to the surveillance system in 2011 (Bureau of Epidemiology, 2011). Thailand has a high reported incidence of HFMD during the rainy season, from June to October, with 18,196-45,961 cases and 2-6 deaths reported per year between 2011 and 2013 (Bureau of Epidemiology, 2013). Lopburi has been a province with an increasing number of cases of HFMD each year and 3 deaths reported between 2011 and 2013 (Bureau of Epidemiology, 2013). King Narai Hospital is a referral center for severe HFMD cases for district and private hospitals in Lopburi Province. In this study we attempted to describe the epidemiology and clinical features of HFMD and explore the risk factors associated with severe HFMD among hospitalized patients at King Narai Hospital, Lopburi, Thailand.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of King Narai Hospital. We retrospectively reviewed the inpatient medical records of HFMD cases among patients aged <15 years admitted to King Narai Hospital between 2011 and 2013 by searching the hospital ICD-10 database, laboratory log book, and HFMD investigation system at the hospital. The ICD-10 codes searches were: B08.4 (Enteroviral vesicular stomatitis with exanthema; HFMD), B34.1 (Enterovirus infection, unspecified), B97.1 (Unspecified enterovirus as the cause of diseases classified elsewhere), A85.0 (Enteroviral encephalitis), A87.0 (Enteroviral meningitis).

Information about the clinical symptoms and signs, duration and severity of illness, length of hospital stay, laboratory results and treatment outcomes were obtained from the medical records.

The patients were classified into 3 groups: 1) A suspected case, defined as a patient with a clinical diagnosis of HFMD; 2) A probable case, defined as a suspected case with the presence of enteroviral specific IgG antibodies with a titer of at least 1:512 on a single serum sample, detected by a micro-neutralization method, because fewer than 6% of healthy children and fewer than 1% of healthy adults have a serum titer ≥1: 512 (Luo *et al*, 2009; Linsuwanon *et al*, 2014); 3) A confirmed case, defined as a suspected case with one of the following: the presence of enteroviral RNA by reverse transcriptase polymerase chain reaction (RT-PCR), the presence of enterovirus by viral isolation, or a four-fold increase in enteroviral specific IgG antibodies titer from samples collected at

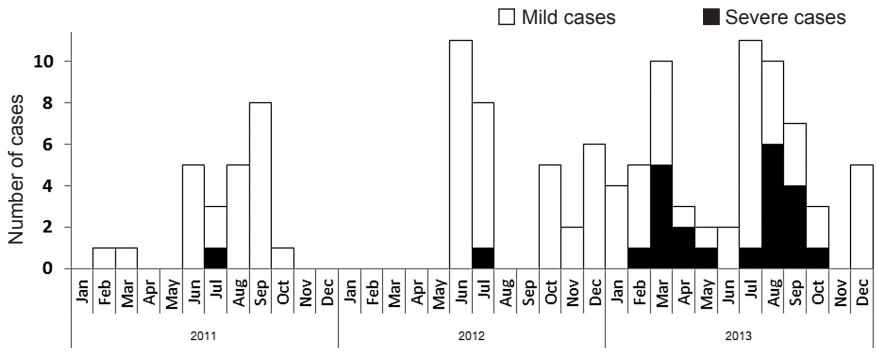

Fig 1–Distribution of hand, foot and mouth disease by severity at King Narai Hospital between 2011 and 2013 (*N*=118).

least two weeks apart and detected by a micro-neutralization method.

All laboratory tests were performed at the National Institute of Health, Department of Medical Science, the Ministry of Public Health, Thailand.

All cases were then categorized by severity into mild or severe. Severe cases were HFMD patients with pneumonia, pulmonary edema, respiratory failure, encephalitis, meningitis, meningo-encephalitis, or myocarditis. Mild cases were patients with HFMD who did not meet the criteria for a severe case.

Data were entered and analyzed with Epi-Info version 7.1.3 (Center for Disease Control and Prevention, Atlanta, GA) associated factors for severe HFMD were analyzed. The chi-square test was used to ascertain differences in signs and symptoms between severe and mild cases. Data were considered statistically significant at a *p*-value <0.05. We calculated the risk ratio (RR) with a 95% confidence interval (CI) on univariate analysis for each variable including gender, age, clinical features, underlying disease, duration of fever, duration of hospitalization, laboratory results, and possible risk for severe enterovirus infection. To adjust for potential confounders, we performed a stepwise multivariate logistic regression analysis by adding variables demonstrating significance on univariate analysis and biological plausibility to calculate the adjusted odds ratio.

RESULTS

A total of 134 medical records of enterovirus infection cases were reviewed, and 118 met the case definition for HFMD and were further analyzed. The male to female ratio was 1.8:1 with a median age of 1 year (range was 25 days to 14 years). Most cases (94%) were children aged <5 years. In 2011, 24 cases were identified with the peak of onset of illness from June to October. The number of cases increased from 32 in 2012 to 62 in 2013, probably due to the continuation of an epidemic beginning in October, 2012 and continuing until March, 2013. The number of severe cases increased from 1 in 2012 to 21 in 2013, which was the year with the highest number of severe HFMD cases in Lopburi (Fig 1).

Of the 118 cases, 27 were confirmed, 4 were probable, and 87 were suspected. Ninety-five cases (80.5%) were categorized as mild and 23 (19.5%) as severe, including 5 fatalities. Of the 27 confirmed

Table 1
Severity and causative agents for hand, foot and mouth disease at King Narai Hospital between 2011 and 2013 (*N*=118).

Severity of cases	Mild	Severe	Total
Confirmed cases	6	21	27
CA16[a]	3	9	12
EV71[b]	2	6	8
EV71 and CA16	0	4	4
Pan-enterovirus (enterovirus which was neither EV71 nor CA16)	1	2	3
Probable cases			
EV71	2	2	4
Suspected cases	87	0	87
Total	95	23	118

[a]One dual infection with CA16 and dengue virus; [b]One dual infection with EV71 and dengue virus.

cases, 12 (44.5%) were infected with CA16, 8 (29.6%) with EV71, and 4 (14.8%) had dual infections with both EV71 and CA16. Three cases (11.1%) tested positive for pan-enterovirus (an enterovirus that is neither EV71 nor CA16). We also identified two cases with dual infection with enterovirus (one with EV71 and one with CA16) and dengue virus.

Of the mild cases, 87 were suspected cases, 2 were probable and 6 were confirmed. Of the 6 confirmed mild cases, 3 were infected with CA16, 2 with EV71, and 1 with pan-enterovirus. The 2 probable cases were infected with EV71 and presented with acute gastroenteritis and HFMD, respectively. Of the 21 confirmed cases in the severe group, 9 cases were infected with CA16, 6 with EV71, 4 with both EV71 and CA16 and 2 cases with pan-enterovirus (Table 1).

Of the severe cases, the 2 probable cases were infected with EV71. One probable case was a 12-year-old boy with meningitis whose EV71 and CA16 titers were 1:512 and 1:256, respectively, 11 days after the onset of symptoms. The other probable case was a 4-year-old boy with diabetic ketoacidosis and encephalitis who had a fatal outcome. The blood specimen collected on day 4 showed a positive titer for EV71 at 1:8,192.

The most common, histories and clinical symptoms and signs in the 118 cases at the time of admission were: history of fever (96%), oral lesions (77%), tachycardia (60%), sore throat (53%), a body temperature of ≥37.8°C on admission (55%), skin lesions (58%), rhinorrhea (43%), and cough (42%). The clinical features found in a higher prevalence ($p<0.05$) mild cases than severe cases were oral lesions, sore throat, skin lesions. Seizures, diarrhea, dyspnea, and drowsiness/lethargy were significantly more common among severe cases (Table 2). The most common manifestations among the 23 severe cases were: seizures (74%), gastrointestinal manifestations (48%), pneumonia (39%), encephalitis (39%), meningitis (13%), meningoencephalitis (9%), and hyperglycemia (9%) (Fig 2). The characteristics of

Table 2
Clinical manifestations by severity of hand, foot and mouth disease cases on admission at King Narai Hospital during 2011-2013.

	Total cases (*N*=118) *n* (%)	Severe cases (*N*=23) *n* (%)	Mild cases (*N*=95) *n* (%)	*p*-value
History of fever	114 (96)	23 (100)	91 (96)	0.32
Oral lesions	91 (77)	4 (17)	87 (92)	<0.001
Tachycardia	71 (60)	17 (74)	54 (57)	0.13
Sore throat	62 (53)	3 (13)	59 (62)	<0.001
Admission temp ≥37.8ºC	65 (55)	13 (56)	52 (55)	0.87
Skin lesions	69 (58)	1 (4)	68 (72)	<0.001
Rhinorrhea	51 (43)	9 (39)	42 (44)	0.66
Cough	49 (42)	12 (52)	37 (39)	0.25
Nausea/vomiting	34 (29)	6 (26)	28 (30)	0.74
Seizures	26 (22)	17 (74)	9 (9)	<0.001
Diarrhea	17 (14)	7 (30)	10 (10)	0.01
Fatigue	9 (8)	3 (13)	6 (6)	0.27
Dyspnea	12 (10)	11 (48)	1 (1)	<0.001
Drowsiness/lethargy	13 (11)	12 (52)	1 (1)	<0.001

the fatalities are shown in Table 3.

The clinical manifestations significantly associated with severe HFMD on both univariate and multivariate analyses were: age <1 year, absence of oral lesions and drowsiness/lethargy (Table 4).

DISCUSSION

An increase in the incidence of severe HFMD and fatalities at King Narai Hospital, Lopburi Province, Thailand was seen between 2011 and 2013. The greatest number of cases (62) occurred during 2013. This reflects the overall trend for Thailand (Bureau of Epidemiology, 2013) with an increasing incidence during the same time period. The increasing trend in HFMD may be due to a change in the case definition within the reporting system, which has included herpangina since September 2011. An increasing awareness among physicians may also have contributed to greater recognition and more testing for enterovirus. We also found a continuation of the epidemic during the winter and summer period from 2012 to 2013, which is an unusual period for HFMD occurrence in Thailand. Global climate change may have played a role in the rising number of cases because an increase in the average temperature and relative humidity was reported concurrently with the increase in HFMD incidence (Onozuka and Hashizume, 2011; Wang *et al*, 2011).

Nineteen point five percent of cases in our study (23/118) were severe; 61% presented with neurological manifestations (encephalitis 39%, meningitis 13%, and meningo-encephalitis 9%) and respiratory involvement (pneumonia 39%). Although the majority of HFMD cases occured in children aged <5 years, most of the severe cases in this study were aged <1 year, especially in the fatalities, which is compatible with other studies (Ni *et al*, 2012). The number of male cases was approximately twice that of female cases

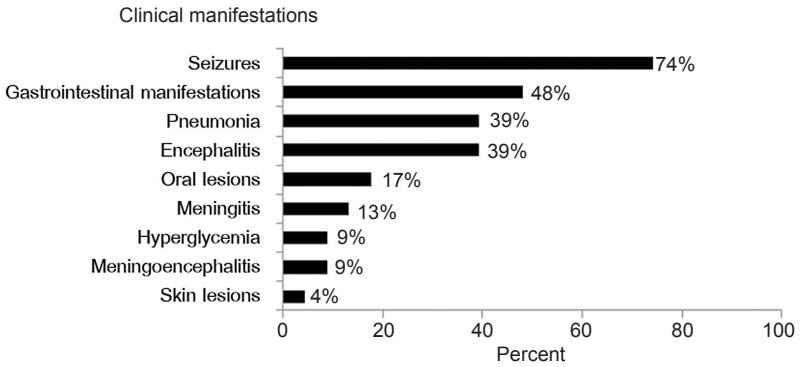

Fig 2–Percentage of patients having specific clinical manifestations of severe hand, foot and mouth disease at King Narai Hospital, between 2011 and 2013 (*N*=23).

for both severe and mild forms, but this difference was not statistically significant, which is consistent with other studies (Suzuki *et al,* 2010; Fang *et al*, 2014).

EV71 was a common cause of severe HFMD in our study, which included 4 fatalities. Many studies have reported that EV71 plays a major role in causing severe HFMD (Chen *et al*, 2007; Liu *et al*, 2012; Gao *et al*, 2014). However, CA16 can also cause severe HFMD (Suzuki *et al,* 2010; Ni *et al*, 2012). We found dual infections with both EV71 and CA16 in 4 confirmed cases. Previous studies have concluded that neurons are the main viral targets for EV71 infections resulting in neurogenic pulmonary edema as the primary cause of death (Li *et al*, 2002; Shih *et al*, 2008). Other enteroviral agents can also cause HFMD epidemics, such as coxsackie A6, which has been reported in Thailand and other countries as the causative agent of HFMD (Wu *et al*, 2010; McIntyre *et al*, 2012; Puenpa *et al*, 2013).

Clinical manifestations associated with severe HFMD among children aged <1 year include: the highest body temperature ≥39.0°C, duration of fever ≥3 days, absence of skin lesions or oral ulcers, diarrhea, dyspnea, drowsiness/lethargy, seizures and hyperglycemia (Chang *et al*, 1999; Fang *et al*, 2014). Tachycardia and leukocytosis have been reported to be significantly associated in some studies (Pan *et al,* 2012; Li *et al*, 2014) but we did not find this in our study. Skin lesions were significantly associated with severe HFMD on univariate analysis only. However, the absence of skin lesions has been found to be related to severe disease in some studies (Zhou *et al*, 2014).

On multivariate analysis, age <1 year, drowsiness/lethargy and absence of oral lesions were the risk factors for severe disease. Chang *et al* (1999) suggested lethargy is a useful clinical symptom of neurological involvement early in the illness. Seizures are a main symptom of neurological involvement and associated with severe disease (Chan *et al*, 2000; Ooi *et al*, 2007). However, in our study neurological signs were not associated with severe disease, unlike in a study from Vietnam (Nguyen *et al*, 2014) that found myoclonus in 66% of cases of severe disease.

Oral ulcers are common in mild disease but rare in severe disease. Some have reported the absence of mouth ulcers is associated with more severe disease and children without mouth ulcers should be monitored closely (Pan *et al*, 2012). Severe disease is characterized by 3 stages: central nervous system involvement, autonomic nervous system dysregulation and cardiopulmonary failure (Huang *et al*, 1999; Lin

Table 3
Case fatalities due to hand, foot and mouth disease at King Narai Hospital between 2011 and 2013.

Sex/age	Diagnosis	Days from onset to death	Days of hospital stay	Type of enterovirus	Case classification
Male 4 years	DKA with encephalitis, brain edema	4	2	EV71	Probable[a]
Male 3 months	Enterovirus infection, acute respiratory failure, severe sepsis, sudden cardiac arrest	4	1	Pan-enterovirus	Confirmed[b]
Male 2 months	Encephalitis, sudden cardiac arrest, pneumonia, acute respiratory failure	4	4	EV71	Confirmed[b]
Female 1 month	Encephalitis, pneumonia, respiratory failure	4	1	EV71 & C A16	Confirmed[b]
Male 25 days	Encephalitis, sepsis, acute respiratory failure	4	1	EV71	Confirmed[b]

[a]A probable case was a suspected case with the presence of viral specific IgG antibodies at least 1: 512 from a single serum sample detected by micro-neutralization.

[b]A confirmed case was a suspected case with one of the following: the presence of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR), the presence of enterovirus by viral isolation, or a four-fold increase in IgG antibodies in samples collected at least two weeks apart and detected by the micro-neutralization method.

Table 4
Univariate and multivariate analyses of various factors and their association with mild and severe hand, foot and mouth disease at King Narai Hospital between 2011 and 2013.

Variables	Severe cases	Mild cases	Total	RR (95% CI)	Adjusted OR (95% CI)[a]
Gender					
Male	16	60	76	1.26	0.84
Female	7	35	42	(0.56-2.82)	(0.04-14.74)
Age					
<1 year	12	8	20	5.34	27.19
≥1 year	11	87	98	(2.75-10.36)	(1.38-534.48)
Pathogen					
EV71	12	4	16	6.95	1.32
Other enterovirus	11	91	102	(3.71-13.00)	(0.06-26.88)
Temperature at admission					
≥37.8ºC	13	52	65	1.06	-
<37.8ºC	10	43	53	(0.50-2.22)	
Highest temperature					
≥39.0ºC	16	21	37	5.00	1.78
<39.0ºC	7	74	81	(2.25-11.11)	(0.10-30.50)
Duration of fever					-
≥3 days	20	49	69	4.73	
<3 days	3	46	49	(1.48-15.05)	
Tachycardia					-
Yes	17	54	71	1.30	
No	6	41	47	(0.96-1.75)	
Skin lesions					
Yes	1	68	69	0.03	0.07
No	22	27	49	(0.004-0.23)	(0.004-1.16)
Oral lesions					
Yes	4	87	91	0.06	0.007
No	19	8	27	(0.02-0.16)	(0.0002 -0.27)
Vomiting					-
Yes	6	28	34	0.86	
No	17	67	84	(0.37-2.02)	
Diarrhea					
Yes	7	10	17	2.59	0.14
No	16	85	101	(1.25-5.34)	(0.005-3.83)
Sore throat					
Yes	3	59	62	0.13	0.75
No	20	36	56	(0.04-0.43)	(0.03-14.95)
Dyspnea					
Yes	11	1	12	8.09	0.17
No	12	94	106	(4.62 - 14.16)	(0.002-11.10)
Drowsiness/Lethargy					
Yes	12	1	13	8.81	179.24
No	11	94	105	(4.92-15.74)	(2.46-13,043.42)

Table 4 (Continued).

Variables	Severe cases	Mild cases	Total	RR (95% CI)	Adjusted OR (95% CI)[a]
Seizures					-
Yes	17	9	26	10.02	
No	6	86	92	(4.40-22.82)	
Hyperglycemia (BS>200 mg%)					-
Yes	6	0	6	6.58	
No	17	95	112	(4.25-10.20)	
Leukocytosis (WBC≥10,000)					-
Yes	19	79	98	0.96	
No	4	16	20	(0.36-2.54)	
Length of hospitalization					-
≥3 days	16	31	47	3.45	
<3 days	7	64	71	(1.53-7.74)	

[a]Variables included in the multivariate model were gender, age, pathogen, highest temperature, skin lesions, oral lesions, sore throat, diarrhea, dyspnea, and drowsiness/lethargy.

et al, 2002). We recommend monitoring closely patients with suspected enterovirus infection without oral and skin lesions, particularly during an epidemic, to look for neurological signs, symptoms of sympathetic hyperactivity and cardiopulmonary involvement.

Of the 5 fatal cases, 4 were aged ≤3 months. Lack of passive maternal antibodies may have been a reason for severe disease in these babies. Approximately 50%-60% of pregnant women had antibody to enterovirus that can be passed to the neonate (Luo *et al*, 2009). The antibody level among neonates declines and disappears by 6 months of age (Luo *et al*, 2009). Several vaccines for HFMD are currently in development and may play an important role for prevention and control of the disease in the future (Hwa *et al*, 2013; Mao *et al*, 2013). Until a HFMD vaccine becomes available, control of HFMD is limited to other public health interventions, such as hand washing, isolation of patients, and improved clinical management of HFMD by early recognition of the disease especially in severe and fatal cases.

A limitation of this study was the small sample size of severe cases. Prospective surveillance is needed for further clarification of the risk factors for severe HFMD.

In conclusion, CA16 and EV71 were the main causative agents of severe disease in our study. High risk factors significantly associated with severe HFMD included seizures, a highest body temperature ≥39.0˚C, duration of fever ≥3 days, absence of skin lesions, diarrhea, dyspnea, hyperglycemia, age <1 year, drowsiness/lethargy and absence of oral lesions. These results provide information for clinicians to point out what patients should be watched more closely. Early recognition of children at risk and prompt treatment are the important for the best management of severe HFMD patients.

ACKNOWLEDGEMENTS

We are grateful to the staff of King Narai Hospital, the National Institute of

Health, the Department of Medical Science, the Department of Disease Control, and the Lopburi Provincial Health Office for their contributions in providing relevant information to document this study. We especially thank Professor Emeritus Dr Chule Thisyakorn for his valuable advice and critical reading of this study.

REFERENCES

Alexander JP Jr, Baden L, Pallansch MA, Anderson LJ. Enterovirus 71 infections and neurologic disease--United States, 1977-1991. *J Infect Dis* 1994; 169: 905-8.

Bureau of Epidemiology, Ministry of Public Health, Thailand. Surveillance system evaluation. Nonthaburi: Bureau of Epidemiology, 2011. [Cited 2015 Jan 20]. Available from: http://www.boe.moph.go.th/files/report/20140320_87651203.pdf

Bureau of Epidemiology, Ministry of Public Health, Thailand. Hand, foot, and mouth disease. *Annu Epidemiol Surveill Rep* 2013. [Cited 2015 Jan 20]. Available from: http://www.boe.moph.go.th/Annual/AESR2013/index.annual/HFM.pdf

Chan LG, Parashar UD, Lye MS, *et al*. Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: clinical and pathological characteristics of the disease. For the Outbreak Study Group. *Clin Infect Dis* 2000; 31: 678-83.

Chang LY, Lin TY, Hsu KH, *et al*. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *Lancet* 1999; 354: 1682-6.

Chen SC, Chang HL, Yan TR, Cheng YT, Chen KT. An eight-year study of epidemiologic features of enterovirus 71 infection in Taiwan. *Am J Trop Med Hyg* 2007; 77: 188-91.

Chong CY, Chan KP, Shah VA, *et al*. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. *Acta Paediatr* 2003; 92: 1163-9.

Fang Y, Wang S, Zhang L, *et al*. Risk factors of severe hand, foot and mouth disease: a meta-analysis. *Scand J Infect Dis* 2014; 46: 515-22.

Gao LD, Hu SX, Zhang H, *et al*. Correlation analysis of EV71 detection and case severity in hand, foot, and mouth disease in the Hunan Province of China. *PLoS ONE* 2014; 9: e100003.

Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; 341: 936-42.

Hwa SH, Lee YA, Brewoo JN, Partidos CD, Osorio JE, Santangelo JD. Preclinical evaluation of the immunogenicity and safety of an inactivated enterovirus 71 candidate vaccine. *PLoS Negl Trop Dis* 2013; 7: e2538.

Li ML, Hsu TA, Chen TC, *et al*. The 3C protease activity of enterovirus 71 induces human neural cell apoptosis. *Virology* 2002; 293: 386-95.

Li Y, Zhu R, Qian Y, Deng J. The characteristics of blood glucose and WBC counts in peripheral blood of cases of hand foot and mouth disease in China: a systematic review. *PLoS One* 2012; 7: e29003.

Li W, Teng G, Tong H, *et al*. Study on risk factors for severe hand, foot and mouth disease in china. *PLoS One* 2014; 9: e87603.

Lin TY, Chang LY, Hsia SH, *et al*. The 1998 enterovirus 71 outbreak in Taiwan: pathogenesis and management. *Clin Infect Dis* 2002; 34 (suppl 2): S52-7.

Linsuwanon P, Puenpa J, Huang SW, *et al*. Epidemiology and seroepidemiology of human enterovirus 71 among Thai populations. *J Biomed Sci* 2014; 21: 16.

Liu LJ, Xu HM, Li XJ, *et al*. Co-detection in the pathogenesis of severe hand-foot-mouth disease. *Arch Virol* 2012; 157: 2219-22.

Luo ST, Chiang PS, Chao AS, *et al*. Enterovirus 71 maternal antibodies in infants, Taiwan. *Emerg Infect Dis* 2009; 15: 581-4.

Mao Q, Cheng T, Zhu F, *et al*. The cross-neutralizing activity of enterovirus 71 subgenotype c4 vaccines in healthy chinese infants

and children. *PLoS One* 2013; 8: e79599.

McIntyre MG, Stevens KM, Davidson S, *et al*. 24.Notes from the field: severe hand, foot, and mouth disease associated with coxsackievirus A6 - Alabama, Connecticut, California, and Nevada, November 2011-February 2012. *MMWR* 2012; 61: 213-4.

Ni H, Yi B, Yin J, *et al*. Epidemiological and etiological characteristics of hand, foot, and mouth disease in Ningbo, China, 2008-2011. *J Clin Virol* 2012; 54: 342-8.

Nguyen NT, Pham HV, Hoang CQ, *et al*. Epidemiological and clinical characteristics of children who died from hand, foot and mouth disease in Vietnam 2011. *BMC Infect Dis* 2014; 14: 341.

Onozuka D, Hashizume M. The influence of temperature and humidity on the incidence of hand, foot, and mouth disease in Japan. *Sci Total Environ* 2011; 410-11: 119-25.

Ooi MH, Wong SC, Podin Y, *et al*. Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. *Clin Infect Dis* 2007; 44: 646-56.

Pan J, Chen M, Zhang X, Chen Y, Liu H, Shen W. High risk factors for severe hand, foot and mouth disease: a multicenter retrospective survey in Anhui Province China, 2008-2009. *Indian J Dermatol* 2012; 57: 316-21.

Puenpa J, Chieochansin T, Linsuwanon P, *et al*. Hand, foot, and mouth disease caused by coxsackievirus A6, Thailand, 2012. *Emerg Infect Dis* 2013; 19: 641-3.

Shah VA, Chong CY, Chan KP, Ng W, Ling AE. Clinical characteristics of an outbreak of hand, foot and mouth disease in Singapore. *Ann Acad Med Singapore* 2003; 32: 381-7.

Shekhar K, Lye MS, Norlijah O, *et al*. Deaths in children during an outbreak of hand, foot and mouth disease in Peninsular Malaysia-clinical and pathological characteristics. *Med J Malaysia* 2005; 60: 297-304.

Shih SR, Weng KF, Stollar V, Li ML. Viral protein synthesis is required for Enterovirus 71 to induce apoptosis in human glioblastoma cells. *J Neurovirol* 2008; 14: 53-61.

Suzuki Y, Taya K, Nakashima K, *et al*. Risk factors for severe hand foot and mouth disease. *Pediatr Int* 2010; 52: 203-7.

Wang Y, Feng Z, Yang Y, *et al*. Hand, foot, and mouth disease in China: patterns of spread and transmissibility. *Epidemiology* 2011; 22: 781-92.

World Health Organization (WHO). A guide to clinical management and public health response for hand, foot and mouth disease (HFMD). Geneva: WHO, 2011. [Cited 2015 Jan 20]. Available from: http://www.wpro.who.int/publications/docs/GuidanceforthclinicalmanagementofHFMD.pdf

Wu Y, Yeo A, Phoon MC, *et al*. The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. *Int J Infect Dis* 2010; 14: e1076-81.

Xu W, Liu CF, Yan L, *et al*. Distribution of enteroviruses in hospitalized children with hand, foot and mouth disease and relationship between pathogens and nervous system complications. *Virol J* 2012; 9: 8.

Zhou HT, Guo YH, Tang P, *et al*. No exanthema is related with death and illness severity in acute enterovirus infection. *Int J Infect Dis* 2014; 28: 123-5.