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Review Article

HAND, FOOT AND MOUTH DISEASE

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EPIDEMIOLOGY

Hand, Foot and Mouth disease is an acute viral illness that primarily affects infants and young children and often occurs in clusters or outbreaks. The major causative agents of Hand Foot and Mouth disease are coxsackie virus A16, Human enterovirus 71 and coxsackie virus A10,of the genus Enterovirus in the family Picornaviridae. HFMD caused by EV71 is associated with severe

Neurological complications and death.EV71 was first isolated from a child with aseptic meningitis. Hand, foot, and mouth disease (HFMD) is a common childhood illness caused by enteroviruses. HFMD outbreaks and reported cases have sharply increased in China since 2008. Epidemiological and clinical data of HFMD cases reported in Henan Province were collected from 2008 to 2013. Clinical specimens were obtained from a subset of these cases. Descriptive epidemiological methods were used to analyze the time, region and population distribution. The VP1 gene from EV71 and CA16 isolates was amplified, and the sequences were analyzed. 400,264 cases of HFMD were reported in this study, including 22,309 severe and 141 fatal cases. Incidence peaked between April and May. Laboratory confirmation was obtained for 27,692 (6.9%) cases; EV71, CA16, and other enteroviruses accounted for 59.5%, 14.1%, 26.4%, respectively. Phylogenetic analysis revealed that EV71 belonged to the C4a evolution branch of C4 sub-genotype and CA16 belonged to subtype B1a or B1b. The occurrence of HFMD in Henan was closely related to season, age and region distribution. Children under five were the most affected population. The major pathogens causing HFMD and their genotypes have not notably changed in Henan. The data strongly support the importance of EV71 vaccination in a high population density area such as Henan, China.(1)

Liu's study aimed to analyze the epidemiology and virology of fatal and nonfatal hand, foot, and mouth disease (HFMD) cases in Mainland China. A total of 10 714 237 survivors and 3046 deaths were reported from 2008 to 2014 June, with a case fatality rate of 0.03%. The morbidity of the survivors increased from 37.6/100 000 in 2008 to 139.6/100 000 in 2013 and peaked in 2012 at 166.8/100 000. However, the mortality varied around 0.03-0.04/100 000 across the time. Most of the survivors were distributed in the southern and eastern China, predominantly in the Guangxi and Hainan Province, whereas deaths were dominant in southern (Guangxi) and southwestern (Guizhou) China. The two groups showed similar seasonal fluctuations from 2008 to 2014, peaking in spring and early summer. Of the total cases, 93.97% were children less than 5 years of age, with those ≤ 2 years old accounting for 60.08% versus 84.02% in the survivor and death groups, respectively. Boys were at higher risk of infection than girls in both groups. Five years of virological surveillance showed that 43.73%, 22.04%, and 34.22% of HFMD cases were due to EV71, CoxA16 and other enteroviruses, respectively. EV71 was encountered in most deaths, with no substantial effect of age, gender, month, and year on incidence. Subgenotype C4a was the prevalent EV71 strain in Mainland China, with no significant difference in the VP1 gene related to virulence between the two groups. In conclusion, based on the largest population study, fatal and nonfatal HFMD cases, mainly caused by C4a of EV71, are circulating in Mainland China with a low-cause fatality rate(2)

Since 2008, Mainland China has undergone widespread outbreaks of hand, foot, and mouth disease (HFMD). In order to determine the characteristics of epidemics and enteroviruses (EV) associated with HFMD in Tianjin, in northern China, epidemiological and virological data from routine surveillance were collected and analyzed. In Tianjin, a persistent epidemic of HFMD was demonstrated during 2008-2013, involving 102,705 mild, 179 severe, and 16 fatal cases. Overall, 8234 specimens were collected from 7829 HFMD patients for EV detection during 2008-2013. Enterovirus 71 (EV-A71) and coxsackievirus A16 (CV-A16) were the dominant serotypes during 2008-2012, and they were replaced by CV-A6 as the major causative agent in 2013. Phylogenetic analysis based on complete VP1 nucleotide sequences revealed that multiple CV-A6 lineages co-circulated in Tianjin, which grouped together with strains from China and other countries and split into two distinct clusters (clusters 1 and 2). Most Tianjin strains grouped in cluster 1 and were closely related to strains from several eastern and southern provinces of China during 2012 and 2013. Estimates from Bayesian MCMC analysis suggested that multiple lineages had been transmitted silently before the outbreaks at an estimated evolutionary rate of 4.10 × 10(-3) substitutions per site per year without a specific distribution of rate variances among lineages. The sudden outbreak of CV-A6 in Tianjin during 2013 is attributed to indigenous CV-A6 lineages, which were linked to the wide spread of endemic strains around eastern and southern China. (3)

PATHOGENESIS

Guan et al. investigated the pathogenic spectrum of enteroviruses associated with hand, foot and mouth disease (HFMD) in Jinan, China. A total of 274 specimens with a clinical diagnosis of HFMD in Jinan from 2009 to June 2012 were used. A GenomeLab™ (GeXP)-based multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay was employed to simultaneously detect 15 serotypes of human enteroviruses: human enterovirus (EV)71; coxsackievirus A (CVA)16, 4, 5, 6, 9, and 10; CVB1, 3 and 5; echovirus (Echo) 6, 7, 11, 13 and 19. Results showed that all samples were enterovirus-positive, with the most common serotypes being EV71 (25.18%) and CVA16 (16.06%), followed by CVA10 (14.23%), CVA6 (7.30%), CVB1 (1.09%), Echo6 (0.73%), CVA9 (0.36%), CVB3 (0.36%) and co-infections (5.11%). CVA10 and CVA6 had the third and fourth highest prevalence of pathogens for HFMD, respec- tively. The most prevalent season for CVA10 was from April to August, with a peak in April; for CVA6 it was from April to August, with a peak in June. This is the first report of the pathogenic spectrum of en- teroviruses associated with HFMD in Jinan using the GeXP-based multiplex RT-PCR assay. These data will provide the scientific evidence for the prevention and control of epidemics, as well as therapy for HFMD patients.(4)

Autophagy is an important homeostatic process for the degradation of cytosolic proteins and organelles and has been reported to play an important role in cellular responses to pathogens and virus replication. However, the role of autophagy in Coxsackievirus A16 (CA16) infection and pathogenesis remains unknown. Here, we demonstrated that CA16 infection enhanced autophagosome formation, resulting in increased extracellular virus production. Moreover, expression of CA16 nonstructural proteins 2C and 3C was sufficient to trigger autophagosome accumulation by blocking the fusion of autophagosomes with lysosomes. Interestingly, we found that Immunity-related GTPase family M (IRGM) was crucial for the activation of CA16 infection-induced autophagy; in turn, reducing IRGM expression suppressed autophagy. Expression of viral protein 2C enhanced IRGM promoter activation, thereby increasing IRGM expression and inducing autophagy. CA16 infection inhibited Akt/mTOR signaling and activated extracellular signal-regulated kinase (ERK) signaling, both of which are necessary for autophagy induction. In summary, CA16 can use autophagy to enhance its own replication. These results raise the possibility of targeting the autophagic pathway for the treatment of hand, foot, and mouth disease (HFMD).(5)

Liu et al. wished to understand the genetic recombination and phylogenetic characteristics of human enterovirus A71 (EV-A71) and to explore its potential virulence-related sites. Full-length genomes of three EV-A71 strains isolated from patients in Chenzhou City (China) were sequenced and analyzed. Possible re-combination events and crossover sites were analyzed with Recombination Detection Program v4. 1. 6 by comparison with the complete genome sequences of 231 strains of EV-A71. Similarly, plot and bootscanning analyses were undertaken with SimPlot v3. 5. 1. Phylogenetic trees based on the sequences of VP1 regions

were constructed with MEGA v5. 2 using the Kimura two-parameter model and neighbor-joining method. Results suggested that recombination events were detected among the three EV-A71 isolates from Chenzhou City. The common main parent sequence was from JF799986 isolated from samples in Guangzhou City (China) in 2009, and the minor parent sequence was TW/70516/08. Intertypic recombination events were found in the C4b strain (strain SHZH98 isolated in 1998) and C4a strain (Fuyang strain isolated in 2008) with the prototype strains of CVA4 and CVA14 in the 3D region. The chi-square test was used to screen-out potential virulence-related sites with nucleotide substitutions of different types of hand, foot, and mouth disease (HFMD) cases using SPSS v19.0. Results suggested that there were no significant nucleotide substitutions between death cases and severe-HFMD cases. Eighteen significant nucleotide substitutions were found between death/severe-HFMD cases and mild-HFMD cases, and all these 18 substitutions were distributed only in P2 and P3 regions. Intertypic recombination among the predominant circulating EV-A71 strains in the Chinese mainland and other EV-A strains probably dates before 1998, and intratypic recombination might have occurred frequently in the HFMD outbreak from 2008 to 2012. Substitutions in the noncapsid region may be correlated with the changes in virulence of EV-A71. These data suggest that researchers should pay more attention to the relationships between substitutions in the noncapsid region and the virulence of the virus.(6)

CLINICAL FEATURES

The activity of hand, foot, and mouth disease (HFMD) in Hong Kong was high in 2010. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) had been performed routinely for diagnosis of enterovirus (EV) infection among hospitals in a geographical cluster. The aim of the study was to describe the epidemiology and the clinical pattern of EV-related hospital admission in 2010, and evaluate the impact of RT-PCR compared to conventional method. This was a retrospective study and patients with laboratory confirmed EV infection were included. Demographic information, clinical features, complications, and laboratory findings were analyzed. Among the 113 patients identified, HFMD was the most common presentation (84/113, 74%), followed by meningitis (10/113, 9%). Respiratory (52/113, 46%) and gastrointestinal symptoms like vomiting (36/113, 32%) and diarrhea (15/113, 13%) were quite common. For cases with central nervous system (CNS) or myocardial complications, there were significantly fewer oral ulcer and rash over hands and feet compared to their counter group. Serious infection mainly affected children younger than 1-year old and adults. Dual infection was noted among seven patients (6%). Compared to RT-PCR, conventional virus isolation detected only 14-16% of the infections. The relative low culture positive rate could be explained by the circulation of Coxsackie A6 in 2010 which was difficult to be isolated by cell culture. Diagnosis of EV infection among hospitalized patients may not be straightforward. EV RT-PCR significantly improved laboratory diagnosis and delineation of epidemiology of EV infection compared to

conventional methods(7)

Of the 100 patients with culture-proven CA9 infections, the mean (SD) age was 4.6 (3.4) years and the male to female ratio was 1.9. For clinical manifestations, 96 patients (96%) had fever and the mean (SD) duration of fever was 5.9 (3.4) days. Sixty one patients (61%) developed a skin rash, and the predominant pattern was a generalized non-itchy maculopapular rash without vesicular changes. While most patients showed injected throat, oral ulcers were found in only 19 cases (19%), among whom, 6 were diagnosed as herpangina. Complicated cases included: aseptic meningitis (n=8), bronchopneumonia (n=6), acute cerebellitis (n=1), and polio-like syndrome (n=1). Phylogenetic analysis for current CA9 strains is closest to the CA9 isolate 27-YN-2008 from the border area of mainland China and Myanmar. The most common feature of CA9 during the 2011 epidemic in Taiwan is generalized febrile exanthema rather than herpangina or hand, foot, and mouth disease. Given that prolonged fever and some complications are possible, caution should be advised in assessing patients as well as in predicting the clinical course.(8)

Six children with aseptic meningitis presented the clinical symptoms and signs of meningitis. Five of them showed subdural effusion and ventriculomegaly, or both on MRI. At follow-ups, neurologic sequel could not be found. Among 24 cases with brainstem encephalitis, there were myoclonic jerks and tremor, ataxia, or both (grade I disease, n = 12), myoclonus and cranial-nerve involvement (grade II disease, n = 4), and cardiopulmonary failure after brain-stem infection (grade III disease, n = 8). In patients with brainstem encephalitis, lesions were predominantly located at the posterior portions of medulla and pons with hypointensity on T1WI and hyperintensity on T2WI. Cerebellar dentate nucleus, caudate nucleus and lenticular nucleus could also be involved. At follow-ups, the patients with mild symptoms had no neurologic sequel and the lesions within brain stem became small or vanished in most cases. While in the majority of serious patients, neurologic sequel could be found and the lesions located at brain stem became encephalomalacia. Fourteen cases with acute flaccid paralysis presented acute limb myasthenia with tendon reflex and muscular tension decreased. On spinal MRI, the lesions predominantly involved anterior horn regions of spinal cord with hypointensity on T1WI and hyperintensity on T2WI. Most patients improved their muscle strength and most lesions of spinal cord became smaller or vanished during follow-upsMRI is the most effective modality of diagnosis and follow-up for neurologic complications in children with enterovirus 71-infected hand-foot-mouth disease. On MRI, the lesions mainly involve the anterior horn of spinal cord, medulla oblongata and pons. At follow-ups, most patients have no neurologic sequel and the visualized lesions will be absorbed after active treatment(9)

Among the 2379 patients, 1798 were common cases and 581 severe cases, 14 of which resulted in death. Most cases were in children younger than 5 years. Morbidity peaked in July and was higher in the surrounding country and cities than in Harbin proper. Medical expenses were significantly higher for severe than for common cases ($P < 0.001$). The primary clinical symptoms were fever and erythema; laboratory

examination showed leucocytosis together with pneumonia, carditis, and abnormal electrocardiogram and electroencephalogram in severe cases. Multivariable Logistic regression analysis showed that the key factors for severe HFMD were age, morbidity location, morbidity area, fever duration, mouth mucosal symptoms, and abnormal serum levels of neutrophils (NEUT), hemoglobin and glucose ($P < 0.05$). To improve prognosis, reduce medical expense and prevent the development of severe cases, we should improve the epidemiological detection of HFMD to treat patients quickly. We should also closely monitor children with the EV71 virus, who present with continuous fever as well as abnormal laboratory results, from areas highly susceptible to HFMD attacks(10)

TREATMENT

Twelve children ages 1 to 5 years and one adult with hand-foot-and-mouth disease were treated with oral acyclovir within one to two days of onset of the rash. Symptomatic relief, defervescence, and significant involution of lesions were seen within twenty-four hours of initiating therapy. Acyclovir was continued for five days, by which time palmar, plantar, and oral lesions were virtually gone. Acyclovir is a molecule tailored to inactivate the thymidine kinase of the herpesvirus. Since the Coxsackie A16 virus causing hand-foot-and-mouth disease lacks this enzyme, the beneficial therapeutic effect must be explained on other grounds, possibly due to enhancement of the antiviral effect of the patient's own interferon(11)

Enterovirus 71 (EV71) is the main causative pathogen of hand, foot, and mouth disease (HFMD). The severe neurological complications caused by EV71 infection and the lack of effective therapeutic medicine underline the importance of searching for antiviral substances. Pyrrolidine dithiocarbamate (PDTC), an antioxidant, has been reported to inhibit the replication of coxsackievirus B (CVB) through dysregulating ubiquitin-proteasome system (UPS). In this study, the authors demonstrated that PDTC exerted potent antiviral effect on EV71. Viral RNA synthesis, viral protein expression, and the production of viral progeny were significantly reduced by the treatment of PDTC in Vero cells infected with EV71. Similar to the previous report about the inhibitory effect of PDTC on UPS, the authors found that PDTC treatment led to decreased levels of polyubiquitinated proteins in EV71-infected cells. The inhibitory effect of PDTC on UPS was further confirmed by the increased accumulation of cell cycle regulatory proteins p21 and p53, which are normally degraded through UPS, while the expression levels of both proteins remained unchanged. We also showed that PDTC had no impact on the activity of proteasome. Thus, we demonstrated that the down-regulation of PDTC on UPS was the result of its inhibition on ubiquitination. More importantly, this study provides evidence that the inhibition on UPS was required for the antiviral activity of PDTC, since MG132, a potent proteasome inhibitor, significantly inhibited the cytopathic effect and viral protein synthesis in EV71-infected cells. The antioxidant property of PDTC did not contribute to its antiviral effect, since N-acetyl-l-cysteine, a potent antioxidant, could not inhibit viral replication. In addition, CPE and viral protein synthesis were not inhibited in the cells pretreated with PDTC 2h before viral infection and then cultured in the media with no

PDTC supplement, while the antioxidant effect of PDTC was retained. PDTC also showed significant inhibition on apoptosis induced by EV71 infection when it was applied at the early stage of viral infection. Our results collectively suggest that PDTC could be a potential anti-EV71 compound which possesses both antiviral and anti-apoptotic capacity.(12)

Enterovirus 71 is one of the major causative agents of hand, foot and mouth disease in children under six years of age. No vaccine or antiviral therapy is currently available. In this work, we found that the number of B cells was reduced in enterovirus 71-infected mice. Deferoxamine, a marine microbial natural product, compensated for the decreased levels of B cells caused by enterovirus 71 infection. The neutralizing antibody titer was also improved after deferoxamine treatment. Furthermore, deferoxamine relieved symptoms and reduced mortality and muscle damage caused by enterovirus 71 infection. This work suggested that deferoxamine has the potential for further development as a B cell-immunomodulator against enterovirus 71. (13)

Even relatively mild HFMD patients without central nervous system (CNS) complications had elevated serum levels of inflammatory cytokines, including interleukin (IL)-3, IL-6, IL-12p40, and tumor necrosis factor (TNF)-α, it suggested systemic inflammation. In contrast, these patients also have decreased levels of other serum biomarkers, including IL-1Ra, IL-8, IL-16, soluble ICAM-1, CXCL-1, and CCL27. The dysregulation of cytokine and chemokine expression may be involved in CNS complications and unbalanced circulating leukocytes in HFMD patients. Surprisingly, patients treated with methylprednisolone had no difference in the expression levels of HFMD-associated biomarkers instead had slightly increased levels of IL-17A, which was not associated with the occurrence of HFMDWhether steroid treatment has any beneficial effect on the prognosis of HFMD patients requires to be further investigated.(14)

PROGNOSIS

Among the 63 children, 43 were boys and 20 were girls, and their mean age was 25±18 months, with 81% under 3 years old. The four death cases were all under three years old. Compared with the cured cases, the death cases had a significantly lower mean age (8±3 months vs 25±18 months; $P<0.05$). Poor peripheral circulation above the elbow or knee joint, pulmonary edema involving at least two thirds of the lung field, and pulmonary hemorrhage were all closely related to death ($P<0.01$). The death cases and cured cases had significantly different peripheral white blood cell counts, blood lactic acid, and blood glucose (24±11×10(9)/L vs 12±5×10(9)/L; 6.6±1.8 mmol/L vs 3.6±1.7 mmol/L; 16.4±2.5 mmol/L vs 10.0±3.0 mmol/L). The cases with critical illness score <90 had a significantly higher death risk ($P<0.01$). Children with critical HFMD are mainly under 3 years old. The children face extremely high risk of death when they suffer from poor peripheral circulation above the elbow or knee joint, pulmonary edema involving at least two thirds of the lung field, and pulmonary hemorrhage. Significant increases in peripheral white blood cell counts, blood lactic

acid, and blood glucose are risk factors for poor prognosis. Critical illness score is also related to poor prognosis.(15)

CONCLUSION

To prevent the outbreak of infectious diseases like HFMD, effective disease surveillance systems would be especially helpful to give signals of disease outbreaks as early as possible. Effective treatment modules are yet to be studied for the better prognosis and treatment of hand foot and mouth disease.

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