Review Article

Hand, foot, and mouth disease: Current scenario and Indian perspective

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ABSTRACT

Hand, foot, and mouth disease (HFMD), first reported in New Zealand in 1957 is caused by Coxsackievirus A16 (CVA16) and human enterovirus 71 (HEV71) and occasionally by Coxsackievirus A4–A7, A9, A10, B1–B3, and B5. This is characterized by erythematous papulo vesicular eruptions over hand, feet, perioral area, knees, buttocks and also intraorally mostly in the children. HFMD has been known for its self limiting course. Only small scale outbreaks have been reported from United States, Europe, Australia, Japan and Brazil for the first few decades. However, since 1997 the disease has conspicuously changed its behavior as noted in different Southeast Asian countries. There was sharp rise in incidence, severity, complications and even fatal outcomes that were almost unseen before that period. Following the near complete eradication of poliovirus, HEV71, the non-polio enterovirus, may become the greatest threat to cause significant neurological complications. This adds to the fact that effective therapy or vaccine is still a far reaching goal. There are reports of disease activity in different corners of India since 2004. Although of milder degree, continuous progress to affect larger parts of the country may indicate vulnerability of India from possible future fatal outbreaks. Low level of awareness among the health care providers may prove critical.

Key words: Coxsackievirus A16, hand foot and mouth disease, human enterovirus 71, India, Southeast Asia

INTRODUCTION

Hand, foot, and mouth disease (HFMD) was first reported in New Zealand in 1957.[1] Coxsackievirus A16 (CVA16) was first identified next year in 1958 in Canada.[1] HEV71 was discovered much later in 1969 in California[2] from the stool of an infant who was suffering from non-HFMD encephalitis. Etiological relation between HFMD and HEV71 was identified for the first time in 1973 in Sweden and Japan.[3]

In contrast to poliomyelitis, another enteroviral disease renowned for its significant neurological complications, HFMD has been considered to be a benign disease of self limiting nature. For this reason, this has got less attention from the medical fraternity, researchers, public health department and policy makers. This is evident from the non-availability of effective vaccines or stringent preventive policy. There is insufficient level of awareness among the practitioners. Now with reports of many fatal attacks in different Southeast Asian countries, it has become a cause of concern.

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CLINICAL PRESENTATION

Children below 10 years of age are the prime target.[4] Generally, the manifestation is limited to the skin. Mild fever and constitutional symptoms may precede or accompany the skin eruptions. HFMD is characterized by sudden appearance of erythematous papulo vesicular eruptions. Vesicles are round or oval. Generally, they appear in crops and persist in groups over some specific

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areas like hand, feet, perioral area, knees, buttocks and also intraorally. Vesicle fluid is initially clear but rapidly becomes turbid mimicking pustules. There is characteristic perilesional erythema. Lesions in thick skin like palms and soles may not develop classical vesicle; they may instead persist as erythematous papules [Figures 1-6]. Disease usually improves spontaneously after 7-10 days without any complication.

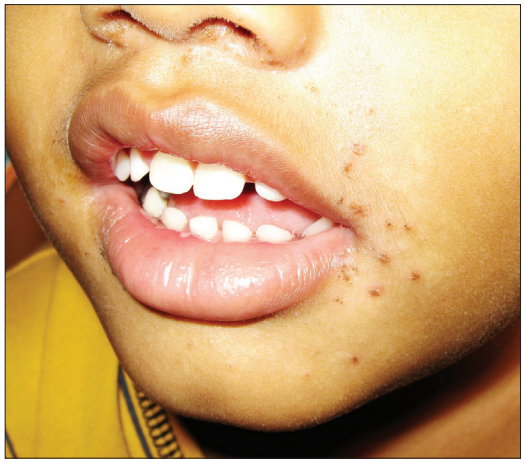

Figure 1: Perioral grouped vesicular eruptions in Hand, foot, and mouth disease

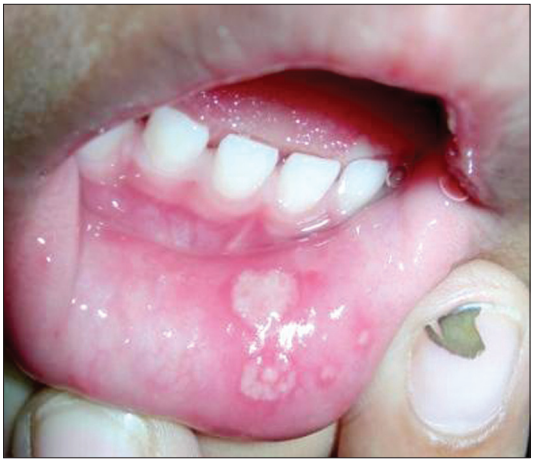

Figure 2: Oral mucosal vesicular lesions in Hand, foot, and mouth disease

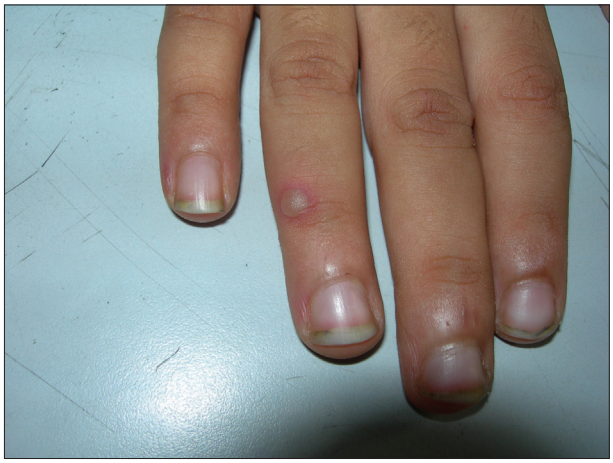

Figure 3: Classical greyish vesicle with erythematous halo on finger

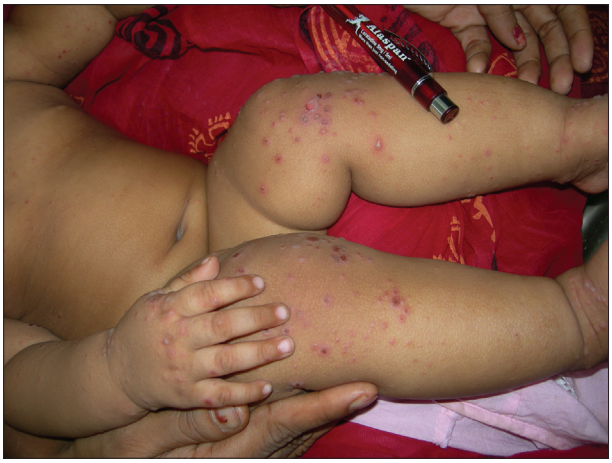

Figure 4: Grouped vesicles on knees in a child

Figure 5: Grouped vesicles on buttocks (Figure 2 and 5 have been reproduced from reference no. 109 with prior permission)

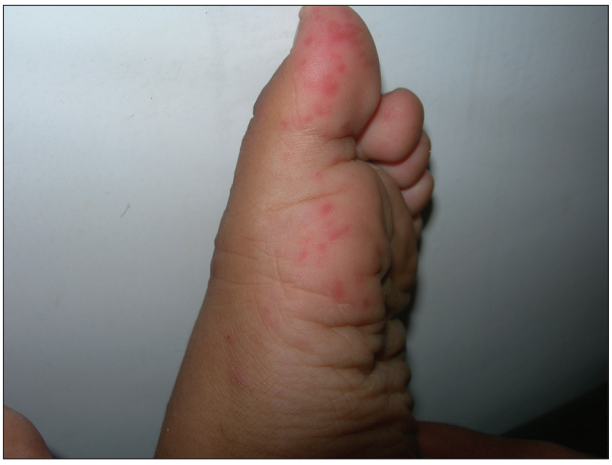

Figure 6: Erythematous papules and ill-formed vesicular eruption on medial margin of foot. (Figure 3, 4 and 6 have been reproduced from reference no. 51 with prior permission)

Skin lesion may resemble chicken pox, herpes simplex (of lip or other area), impetigo, pompholyx, papular urticaria, insect bite and some bullous disease like chronic bullous disease of childhood (CBDC).

Herpangina, another manifestation by the same organisms, is characterized by more limited involvement with ulcers over anterior tonsillar pillars, soft palate, buccal mucosa, or uvula without any skin lesions.

In severe disease, cardiorespiratory and neurological involvement may develop. Cardiopulmonary involvement indicates more severe disease and may herald fatality. Tachycardia, dyspnea, tachypnea, poor peripheral perfusion indicate involvement of cardiopulmonary system. Refractory cardiac dysfunction and fulminant pulmonary oedema may lead to abrupt death. It is said that the rapidity of event usually stuns the primary medical facility providers especially if they are not very much expectant and prepared to manage such situations.[5-8]

Neurological involvement ranges from aseptic meningitis, encephalitis, acute flaccid paralysis (AFP) and manifest with headache, irritability, stiff neck, lethargy, drowsiness, coma, seizures, myoclonus, and limb weakness to complete paralysis. Autonomic dysfunctions like neurogenic bladder, insomnia, profuse sweating, and unexplained transient hyperglycemia may also occur in HEV71 induced cases.[7]

Neurologic involvement, through development of 'neurogenic pulmonary edema' is often the reason behind the cardiac and pulmonary derangement that may ultimately lead to death.

An atypical phenotype of the disease was noted among fatal cases during the epidemic of Sarawak in 1997. These cases had predominant presentation of neurological disease. Similar clinical phenotype was observed in Taiwan in 1998 and Fuyang district, China in 2008. Even before the diagnosis was suspected as HFMD, large number of cases died. Surprisingly, all these three epidemics were primarily caused by HEV71.

During the fatal epidemic of Taiwan in 1998, many cases developed brainstem encephalitis that was classified into three grades (I to III).[9] Most advanced stage (grade III) severely damaged the medulla oblongata, pons and midbrain structures leading to a condition described as 'neurogenic pulmonary edema'.[7,9,10] Neurogenic pulmonary edema is now known as the most dreaded complication that has caused large number of fatalities in Southeast Asian countries in last two decades. Even all the fatalities in Bulgarian epidemic were also supposed to be caused by this. Clinical diagnosis of this condition may be difficult as it may clinically mimic acute myocarditis.[9,10]

It has been shown that pleocytosis in CSF may be a helpful guide to predict sudden death due to cardiorespiratory failure,[6,11] even without obvious neurological symptoms. Presence of intense inflammation in the midbrain has been repeatedly detected in post-mortem histology of the midbrain structures among cases who died of neurogenic pulmonary edema.[12] This is in fact a condition with extremely poor prognosis where mortality may reach up to 80%[7] and may result in high morbidity among the survivors.[13]

Prognosis may not be predicted correctly, especially early in the course. Presence of some atypical physical findings like tachycardia, tachypnea, hypotension, hypertension, bleeding in GIT and neurological deficits, elevated leukocyte count, vomiting and absence of mouth ulcers are reported to have some predictive value.[14]

THE ORGANISMS AND ITS INTERPLAY WITH THE HOST

Coxsackievirus A16 (CVA16) and human enterovirus 71 (HEV71), the most common organisms of HFMD are members of Enterovirus genus,[1] family *Picornaviridae*. HEV71 is a small, non-enveloped, positive-stranded RNA virus. Simultaneous presence of both has also been reported.[2,15,16] Other than these two organisms, Coxsackievirus A4–A7, A9, A10, B1–B3, and B5 have also been reported as relatively rare etiologic agents.[17,18]

HEV71 has four capsid proteins VP1, VP2, VP3 and VP4. These play role in adsorption and uncoating of the virus in the infected human cells. Among these, VP1 is the most pathogenic and crucial one.[19] Thus this has been a good target antigen for preparing an effective subunit vaccine.[20] Based on the highly variable genetic sequences of the *VP1* capsid antigen gene, HEV71 has been classified into different genogroups (A, B, C and D).[21,22] Each genogroup has

many lineages like B0-5, C1-5.[21,23-25] As per the latest report, genogroup D consist of only single strain.[22] Within the same genogroup, there is more than 92% nucleotide sequence identity that is much higher than the rate (78–83%) in other groups.[26]

So far, only two types of receptor for viral entry into the cell is identified. These are human P-selectin glycoprotein ligand (PSGL)-1 and scavenger receptor (SCAR) B2.[27,28] Identification of receptors are useful in understanding the disease pathogenesis because the distribution of the receptors signifies the susceptible cells of the human body.

Neurological involvement is frequently noted in HFMD associated with HEV71 but this is very unusual in CA16 associated cases.[29] This fact is unexplained and may be due to possible existence of a specific HEV71 receptor on neuronal cells. However, such receptors remain unidentified.

THE GROWING CONCERN

For about 3 decades following its discovery, only small scale outbreaks have been reported from United States, Europe, Australia, Japan and Brazil.[2,30-35] Larger outbreaks with high mortality were almost unseen except the outbreak that occurred in Bulgaria in 1975[36] and Hungary in 1978[37] and caused high mortality. However, shift in the behavior of the disease since its entry into Southeast Asian countries was quite conspicuous. There have been many hypotheses to explain this as discussed later.

DISEASE IN SOUTHEAST ASIA

The first report of occurrence of HFMD in mainland China dates back to 1981.[1] The etiologic agent was identified as CVA16 in the stool specimens in Xiamen City in 1983. The disease subsequently affected other areas of China. Outbreak HEV71 occurred in Wuhan City in 1987.[38] In ensuing years, the disease became a regular visitor in many parts of China with larger capacity. It showed a tendency to affect mostly the children less than 5 years of age.[3]

Disease started to show its presence in different Southeast Asian countries like Singapore,[14] Vietnam,[17] Taiwan,[39] China,[40] Japan,[41] Malaysia,[42-44] South Korea,[43] and recently in India.[44]

During the last two decades, there was a sharp rise in incidence, severity, complications and fatalities in the Southeast Asian countries.[45] Mortality has been reported to be increased to a whopping 156% over the last years as reported from China.[46] Fatal outbreak occurred in Malaysia (Sarawak) in 1997,[42] Taiwan in 1998[39,47] and Singapore in 2000.[14] There are recent reports of large epidemics with significantly fatal outcome from the World Health Organization's Western Pacific Region.[48-50] Most severe disease outbreak, so far the highest in the world, occurred in China in 2008 that resulted in largest number of complication and fatality.[1] WHO has published reports regarding the growing threat of HFMD.[51] The disease that kept a low profile for long became a cause for concern.

The Chinese outbreak of 2008 was reported to be an ongoing epidemic that has already caused 1200 cases of brainstem encephalitis and 193 deaths.[1]

An unexplained tendency of gradual shift towards HEV71 was noted. This was correlated with progression of severity as well. Coxsackievirus was detected in the non-fatal outbreaks in 1996 and 1997 but HEV71 in fatal outbreak in 1998 in Taiwan.[11] Similar things happened in China also.

INDIAN PERSPECTIVE

India having a population of more than 1.2 billion, being the 2nd largest country in south East Asia and a close neighbour of China, the worst affected country in the world, had no evidence of the disease till recently. The first report of disease outbreak in India came in 2004 from Calicut.[52] After 3 years, the first large scale outbreak occurred in 2007 from Kolkata and surrounding areas of the eastern state, West Bengal.[44] Since then, many small scale outbreaks have been repeatedly reported from different places.[53-57]

All previous cases of severe outbreaks followed many years of milder attacks, intermittent periods of quiescence and progressively larger areas of involvement. The disease was present in Taiwan since 1980 with only sporadic attacks of mild nature before culminating in a fatal outbreak in 1998.[39,47] Showing the first appearance in 1981, disease caused many progressively larger attacks in China before culminating in the worst ever outbreak in 2008.[1]

Although, no cases of neurological or pulmonary manifestations were detected so far and all the cases

improved spontaneously without any requirement for hospitalization, continuous spread of the disease over larger parts of the country reminds the pre-epidemic periods of China and Taiwan [Table 1]. This is complicated with the extremely low level of awareness among the health care professionals especially the primary level staffs who generally take the most significant step in curbing a severe outbreak.

VARIOUS FACTORS REGULATING SEVERITY

Factors that may play an important role in inducing severity are virological factors, host factor and environmental factors.

Virological factors

HFMD caused by CA16 is relatively milder.[59] In contrast, the disease caused by EV71 is generally more severe[17,39,60] with higher chances of serious complications like myocarditis, neurological involvement like aseptic meningitis, encephalitis, and poliomyelitis-like paralysis.[10,14,61,62] Pulmonary edema is frequently reported from HEV71 associated HFMD.[43,62] For unknown reason, HEV71 associated HFMD has a propensity to infect mostly the children, especially in the 0-24 months age range.[17,50]

Difference in the viral strain may be crucial in determining the disease severity. The strain involved in the outbreaks in China was detected to be only of C4 genotype[58]

These viruses are known to induce mutation to form newer genetic strains following genetic recombination to escape the existing immunity. This may result in re-emergence of the virus.[26] Genotype replacement may occur even during a large epidemic and even between HEV71 and CA16.[63-65]

Since 1997, the most frequently detected strains in south-east Asia are B3-5 and C3-5.[26,66], The single strain in genogroup D was isolated from India in 2002.[22]

Host factors

As mentioned, the striking difference in the disease severity of the disease in many Asian countries hinted at strong possibilities of susceptibility of this population incurred upon by their genetic make-up. *HLA-A33* haplotype, glucose-6-phophate dehydrogenase deficiency,[67] a specific cytotoxic T lymphocyte antigen haplotype (CTLA-4) and some inflammatory cytokines have been proposed as important susceptibility inducing factors. Prevalence of *HLA-A33* haplotype is significantly higher in the Asian populations (17–35%) in comparison to the Caucasian populations.[43] HLA-A 33 (class I) and HLA-DR17 (class II) may also contribute to the severity of the disease caused by HEV71 infection.

Table 1: Some comparative details on the fatal south east Asian HFMD epidemics and the Indian epidemic

Year	Sarawak, Malaysia[6,42] 1997	Taiwan[39-47] 1998	China[1] 2008	India[44] 2007
Age	Among the 29 who died, all were children, median age 1.5 years	87% were <5 years old.[11]	<5 years[40]	Mean 3.4 years
Organism	HEV71	HEV 71	CA16 and HEV71 both were detected.[1] Most severe attacks were due to EV71[40]	Not investigated
Symptoms of severe cases	Classical in mild cases Neurological and cardiorespiratory symptoms in severe cases	Classical in mild cases Neurological and cardiorespiratory symptoms in severe cases	Classical in mild cases Neurological and cardiorespiratory symptoms in severe cases	No neurological and cardio-respiratory symptoms noted in any Fever, anorexia diarrhea was most common symptom
Total cases, Severe case Death/fatality	Total reported cases 2628, Hospitalized -889 Aseptic meningitis or acute flaccid paralysis-39 Death-29	Severe neurologic complications and/or pulmonary edema-405 Death – 77[11*]	Brainstem encephalitis-1200[26] Total case-3.4 million Fatalities-400[58]	38 cases reported from 4 centres No reports of hospitalisation or fatality
Cause of death.	Progressive cardiac failure and pulmonary edema	Progressive cardiac failure and pulmonary edema	Progressive cardiac failure and pulmonary edema	No fatality

*Note: The figure mentioned here reflected the published one. These reflected only the tip of the ice-berg. True figure must be higher.[11] This seems logically applicable to all the figures

Severity of the disease may correlate with the degree of response of some of the indices of cellular immunity like antigen-specific Th1 cytokines and lymphocyte proliferation against HEV71 antigen.[68]

As reported, severe HEV71 encephalitis may have intimate relation with a specific cytotoxic T lymphocyte antigen haplotype (CTLA4).[69] A direct correlation has also been reported with certain inflammatory cytokines in serum and cerebrospinal fluid [10–12].[70-73] Neutralizing antibody levels was not found to have any direct correlation with the severity of the disease caused by HEV71.[46]

DIAGNOSIS

HFMD is generally easily diagnosed on clinical grounds. Although, this shares some clinical resemblance with other diseases like varicella zoster, papular urticaria, impetigo and pompholyx, the constellation of features are unique enough to aid instant clinical diagnosis with certainty in almost all cases. Presence of the disease is usually noted in the surrounding neighbourhoods or schools. Laboratory confirmation is mostly necessary for research purpose, strain analysis or occasionally in cases with atypical manifestations.

Laboratory confirmation can be done directly through the identification of the virus in culture or indirectly through detection of neutralizing antibody in the serum. The last one is particularly helpful in retrospective evaluation of seroprevalence of the disease in the community.

Culture

Stool, throat swab, vesicle fluid can be used for culture. Stool is considered the most appropriate sample owing to its capacity to keep the virus alive for longer duration.[74] It is necessary to keep the transport time as short as possible.

Culture of the organism allows identification of the specific virus through observation of the cytopathic effect in cell culture or formation of plaques in a cell monolayer (plaque assay).[75-77]

HEV71 is known to infect a wide range of cell lines like human RD cells, vero cells, simian virus 40-transformed African green monkey kidney cells (Cos-7), human colorectal carcinoma cells (Caco-2), human pulmonary adenocarcinoma cells (A549), human rhabdomyosarcoma and human embryonic kidney cells, HeLa cells, MRC-5 cells, human immature dendritic cells, human glioblastoma cells (SF268) and human neuroblastoma cells.[1] RD cells is a better cell line for culture than the standard cell line like GMK and L20B cells.[74] Any single cell line cannot grow all the human enteroviruses.[78] Apart from identification of the virus, functional aspects of the virus like replicative fitness and fidelity can also be assessed in culture.[79-81] Its neurotropic effects can be analyzed through its cytopathic effect and neurotoxic mediator release (Cox-2 and PG 2) in human neuroblastoma (SK-N-SH) cell line culture.[82] Nucleotide sequencing of VP1 and VP4 genes can be done for identification of involved strain.[45,83] Another method of identification is fluorescence resonance energy transfer system for HEV71 detection in HeLa cells.[84]

Neutralizing antibody detection

Neutralization with serotype-specific antisera is done to identify the involved serotypes. Plaque reduction neutralization test is generally followed for detection of neutralizing antibody of HEV71.[85]

Heated serum at 56°C for 30 min is serially diluted with 50% tissue culture infective doses of HEV71. It is incubated for 2 h at 37°C in specific cells for 2-7 days. The dilution of plasma that induces cytopathic effect in ≥50% of the tissue culture wells is called the neutralization titre (the reciprocal value).[86,87]

The most important role of detection of neutralizing antibody is evaluation of seroprevalence of the organism in the community. It indirectly reflects the susceptibility of the individual to the organism.

Enzyme-linked immunosorbent assay

In contrast to the culture, RT-PCR or neutralization assay, that are expensive, time consuming and not suitable for use in large mass and in developing country, IgM ELISA has been utilised for rapid diagnosis. It has been shown to be useful with high degree of sensitivity from 1st week till many weeks after infection.[88-91]

Reverse transcriptase–polymerase chain reaction

A recent addition to the diagnostic tests is reverse transcriptase–polymerase chain reaction (RT-PCR) amplification and nucleotide sequencing of the VP1 gene. Reverse transcription polymerase chain reaction (RT-PCR), a variant of polymerase chain reaction (PCR) is a sophisticated and highly sensitive

technique where very low copy levels of RNA is reverse transcribed into its DNA complement (*cDNA*) with reverse transcriptase. PCR then amplifies the cDNA. Among many other uses, this technique is commonly used in diagnosis of viral infections that are caused by RNA viruses like enteroviruses.

RT-PCR is now considered as the primary modality for enterovirus "serotype" identification[16,92-94] replacing the neutralization technique in this regard.[95] This was further improvised to introduce multiplex RT-PCR assay to allow amplification of several RNA viral targets in a single reaction. This can screen multiple pathogens in a single reaction, thus can be a cost effective, as well as rapid screening method[95-97] [Figure 7].

In resource poor set up as in India, laboratory confirmation is generally difficult. Among the seven reports, laboratory confirmation was performed in only two. In one of them, diagnosis of all 4 cases was done clinically and laboratory confirmation through RT-PCR was done in only one case.[57] It detected presence of CA16. In one series, although all those cases improved spontaneously without any fatality, all the 19 cases (100%) were confirmed through microneutralization test in cell culture to be infected with EV71.[52] Phylogenetic analysis was not done so far in any of these Indian cases.

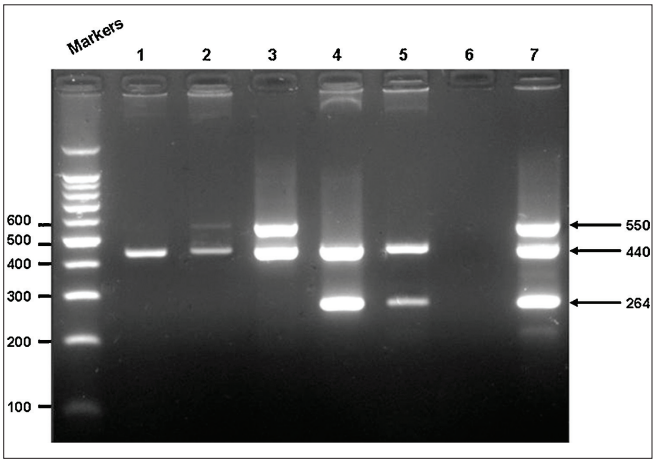

Figure 7: Results of multiplex RT-PCR from a representative sample of throat swab specimens obtained from the HFMD cases:. Lane 1 –enterovirus (unknown serotype); Lanes 2 and 3 –CVA16; Lanes 4 and 5 –HEV71; Lane 6 – negative control; Lane 7 – positive control (a mixture of infected HEV71- and CVA16-infected cell RNA templates). Pan-enterovirus RT-PCR amplicon size – 440 bp; HEV71-specific RT-PCR amplicon size – 264 bp; CVA16-specific RT-PCR amplicon – 550 bp. Molecular weight markers are the 100 bp DNA ladder (Promega). (Figure 7 and legend reproduced from reference no. 95 with prior permission)

MANAGEMENT

There is neither an effective antiviral therapy nor an effective vaccine available against the disease. This is a contagious disease and has the potentiality to spread very fast over a large population in the community. Prevention of further spread of the disease is the only way to control a disease from becoming a large outbreak. As the organisms are enterovirus, they spread through faeco-oral route. Strict implementation of basic protocols like monitoring cleanliness of the hands, utensils and drinking water and avoiding direct contact with affected people can be rewarding. Restriction of the children from attending schools or other outdoor activities is a very simple but effective strategy.

To prevent disease outbreak, disease surveillance, both clinical and laboratory, is useful as found previously in other countries.[17,98,99] Utilizing the information thus obtained can assist the health care authorities to implement early control measure and reduce neurological complication.

Vaccination, currently at research level can be an excellent option for protection of the community. The vaccination has been proposed to be best given at 9 months of age because the maternal protective antibody (Neut-Ab to EV71) wanes off by 6 months of age.[86] As both humoral and cell mediated immunity play definite role in the disease, ideally both should be targeted. Large number of research works are underway, especially against HEV71, the more fatal one.

Inactivated vaccines have been shown to confer satisfactory protection. Formaldehyde inactivation is the most commonly used method for inactivation. Formaldehyde-inactivated whole virus vaccine from a mouse-adapted strain of a genotype B3 clinical isolate was reported to confer significant cross protection against different genotypes. However, there are chances that the inactivated whole virus may regain its virulence if the mutations that are induced by the attenuation process are reversed in the human body. Other ways to induce attenuation have also been tried like inclusion of mRNA homology sequences.[100]

Candidate live-attenuated enterovirus vaccines have largely solved the problem of regaining the virulence. Subunit vaccines[101-103] have also been evaluated but the protective efficacy of these vaccines is yet to be

confirmed.[63,65,89,103-106] Monoclonal antibody belonging to isotype IgM reported to have strong neutralizing activity in mice targeting epitope on VP1 capsid protein has raised some hope.[20]

CONCLUSION

Considering the impact of the severe form of disease, HFMD deserves special attention. Following the near complete eradication of poliovirus, HEV71, the non-polio enterovirus, may become the greatest threat to cause significant neurological complications. This adds to the worry that effective therapy or vaccine is still a far reaching goal.

Monitoring of the disease is required to predict the disease behavior so that control measures can be effectively planned to abort or halt a prospective fatal outbreak. HFMD is classified as category C notifiable infectious disease by the Ministry of Health of China on May 2, 2008.[1] This has also been declared as notifiable diseases in Croatia[74] and Singapore[107] as well. Unfortunately, awareness level in many South-East Asian countries including India is far from the expected level. Research work from Indian subcontinent appears scant.[108] Institution of strict preventive policies, training of health care professionals, initiation of mass awareness programs and encouraging basic and molecular level research activities on this disease seems urgently necessary.[109]

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Multiple Choice Questions

1. Hand foot and mouth disease (HFMD) is not caused by
   a. CV A16 b. CV B1
   c. CV B2 d. CV B 5

2. Presentation of HFMD includes all except
   a. Involvement of buttock is rare b. Perilesional erythema is common
   c. Vesiclulation is always seen d. Disease persist for 3-4 weeks

3. Tick the right answer
   a. Cardiorespiratory and neurological complication is common
   b. Neurogenic pulmonary edema is the predominant mode of death
   c. Major cause of death in HFMD is CV A16
   d. Adults are more commonly affected than children

4. Predictors of sudden death are all except
   a. Pleocytosis in CSF even without obvious neurological symptoms
   b. Atypical physical findings like tachycardia, tachypnea, hypotension, hypertension
   c. Absence of mouth ulcers
   d. Absence of vesicle

5. Post mortem examination of patients died from neurogenic pulmonary edema showed presence of intense inflammation in the
   a. Medulla b. Midbrain
   c. Cerebellum d. Occipital lobe

6. Tick the right answer
   a. HFMD is caused by virus from *Picornaviridae* family
   b. HEV71 is a small, non-enveloped DNA virus.
   c. Genogroup D consist of four strains
   d. Receptors for viral entry into the cell are 3 types

7. Most appropriate sample for diagnosis of HFMD is
   a. Stool b. Blood
   c. Urine d. Skin biopsy

8. Proposed factors for higher susceptibility towards HFMD of the south East Asian population may be due to all except-
   a. *HLA-A33* haplotype,
   b. Glucose-6-phophate dehydrogenase deficiency
   c. Specific cytotoxic T lymphocyte antigen haplotype (CTLA-4)
   d. Socioeconomic status

9. Tick the right answer
   a. Urine is commonly used for virus isolation
   b. HEV71 have been classified into 3 different genogroups
   c. The most pathogenic capsid protein in HEV71 is VP1
   d. Neutralizing antibody (HEV71) directly correlates with the disease severity

10. Tick the wrong one
    a. The strain isolated from India in 2002 was genogroup D
    b. Strain identification can be done with nucleotide sequencing of VP1 and VP4 genes
    c. Rapid diagnosis can be done with IgM ELISA and multiplex RT-PCR assay
    d. Neutralization antibody is at present the technique of choice for serotype identification

Answers
1. c, 2. b, 3. b, 4. d, 5. b, 6. a, 7. a, 8. d, 9. c, 10. d