Review Article

Hand, Foot, and Mouth Disease (HFMD) in India: A Review on Clinical Manifestations, Molecular Epidemiology, Pathogenesis, and Prevention

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Abstract

HFMD is a childhood viral disease initiated by enteroviruses (EVs). Symptoms are initiated with mild-to-moderate fever of short duration followed by oral and skin lesions. Skin lesions are papulovesicular which appears on palms/soles of feet, hands, knees, and elbows. Oral lesions appear as vesicles producing multiple small superficial ulcers. Disease is usually mild illness but sometimes progresses in severe form as meningitis, encephalitis, and polio-like paralysis. Etiological agents of the disease belong to *Picornaviridae* family. The causative viral agents are from genus human enterovirus (HEV) such as enterovirus-A 71 (EV-A71), coxsackievirus –A6 (CV-A6), CV-A10, CV-A16. Coxsackievirus A-16 (CV-A16) and enterovirus A-71 (EV-A71) are the major etiological agents of this disease, among children reported globally. In India, studies conducted on HFMD cases revealed CV-A16 as a major EV type and under circulation over a period of time. Molecular studies of different CV-A16 isolates and the viral kinetic studies conducted on organ tissues of experimental mouse model with complete VP1 gene sequencing revealed presence of B1c sub genotype which is currently in circulation. Genetic changes observed at nucleotide and amino acid level in vital organs of experimental infected mice model might predict some targets and can act as markers of virulence. Mice infected with CV-A16 strains revealed progressive pathological changes in mice organs. Major affected organs were to be as brain, heart, intestine, and skeletal muscles. The present review focuses on HFMD caused by CV-A16 with epidemiological, molecular, pathogenesis and need of antivirals against the disease.

Keywords: *Coxsackievirus A-16, enterovirus A-71, HFMD*

Introduction

HFMD is a common childhood viral infection generally occuring in children under five years.[1] The disease is an illness associated with vesicular lesions of the hands, palm, feet, sole, mouth, and buttocks.[2] It is clinically characterized by a brief febrile illness followed by pharyngitis and rashes on skin. It is typically a mild illness in economically developed countries, and most of the patients usually recover quickly. HFMD occurs globally, and in temperate climates spreads more easily in summer and early autumn. It is common among infants and children below 10 years of age, but can also occur in adults. It is moderately contagious, spreading by direct contact with nose and throat discharges, saliva, fluid from blisters, or the stool of infected persons. Poor hygienic conditions and social contacts are associated with the development of HFMD.[3] A person is most contagious during the first week of the illness, and the disease occurs as both outbreaks and sporadic forms.[4]

Clinical Features

Clinically, the skin rashes are papulovesicular and affect the palms or soles of the feet or both. In some of the patients, rashes may be maculopapular without vesicles and may also involve the buttocks, knees, elbows particularly in younger children and infants. The most common clinical problem associated with HFMD is dehydration, a result of inadequate intake of fluid secondary to odynophagia caused by painful mouth ulcers.[5] Viral incubation period ranges from 5 to 7 days. Illness begins with a mild fever of short duration followed by oral and skin lesions. Oral lesions appear as vesicles, which rapidly ulcerate producing multiple small superficial ulcers

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with erythematous halos. The ulcers resemble aphthous ulcers and are usually seen on the tongue, palate, buccal mucosa, gums, and lips. Skin lesions are vesicles on erythematous bases similar to lesions of varicella but are more elongated and oval. The lesions are present prominently on the hands and feet [Figure 1]. Sometimes, the exanthema is more widespread involving the buttocks, knees, and elbows.[6] Onychoptosis (nail shedding) is also reported to be one of the characteristic features of HFMD.[7,8] Lesions usually subside in 5–7 days. HFMD is usually a mild illness but sometimes may progress to cause meningitis, encephalitis, and polio-like paralysis. Diagnosis of HFMD is usually made based on clinical characteristics but can be confirmed by viral isolation from clinical specimens, such as stool, rectal swab, vesicular fluid, and throat swab.

Most severe complications involve central nervous system (CNS), like aseptic meningitis, acute flaccid paralysis, and encephalomyelitis with or without muscle weakness. Further damage of brainstem results in autonomic dysregulation, pulmonary edema, and myocarditis which may lead to death. Most of the recovered severely affected HFMD cases develop neurological sequelae like cognitive and motor disorder.[9] A large number of mortalities have been reported during severe attack of HFMD outbreaks which occured in Asian countries.[6] About 10–30% of the hospitalized cases having EV-A71-associated HFMD epidemics reported in Asia developed a spectrum of central nervous system (CNS) complications with aseptic meningitis, encephalitis, and acute flaccid paralysis.[10,11] Susceptibility and severity of HFMD are associated with lower age-, which revealed the average to be less than five years of age.[12]

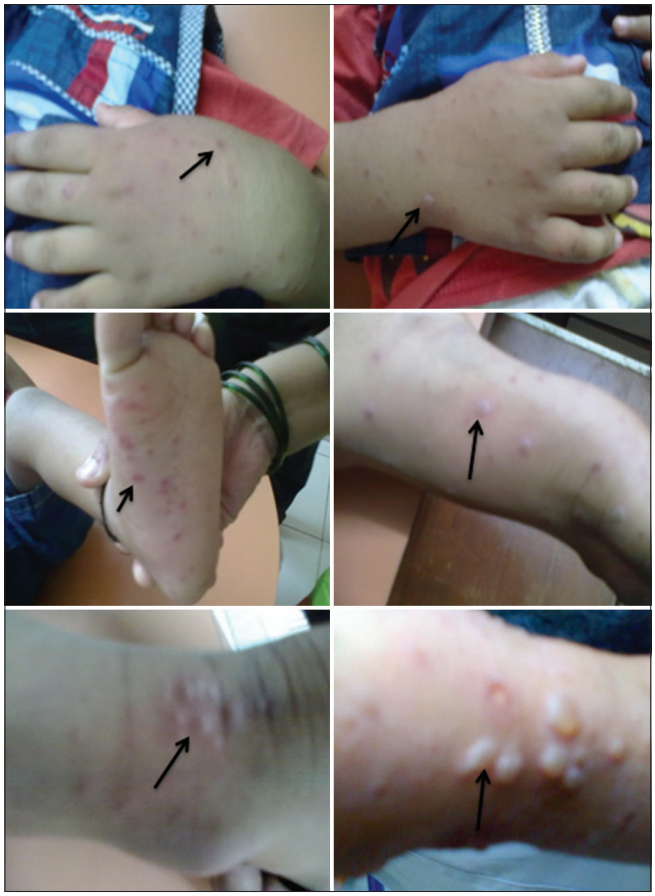

Figure 1: Patient presenting with vesicles and rashes on foot, hand, and sole (indicated with arrows)

Etiology

Human enteroviruses (HEVs) of the family *Picornaviridae* belonging to HEV-A species have been reported to be associated with HFMD. Among EVs, CV-A16 and EV-A71 are the major etiological agents causing HFMD outbreaks. Several outbreaks of the disease have been reported in many parts of the world including Japan,[13] USA,[14] Australia,[15] England, and[16] Singapore,[17] and CV-A16 was found as the main etiological agent. Apart from CV-A16, other coxsackieviruses have also been implicated in various outbreaks including Coxsackievirus A4–A7, A9–A10, B1–B3, B5, and E9 {A4–A7, A10 (HEV-A); A9, B1–B3, B5, E9 (HEV-B)}.[18,19]

Etiological relation of HEV-A71 and HFMD was identified for the first time in 1973 in Sweden and Japan.[20]

Epidemiology

CV-A16 was first identified in 1957 in Canada from an outbreak in a housing estate in Toronto, where 60 patients from 27 families were isolated from a high percentage (71%) of the patients.[21] Thereafter, several outbreaks caused due to CV-A16 and EV-A71 as viral etiological agents were reported from Brunei, Darussalam, China, Malaysia, and Japan. In the year 1981, Singapore reported HFMD cases with involvement of CV-A16 virus[22] and subsequently, Australia,[15] Korea, and Singapore.[17] Sarawak in 1997 had experienced a major outbreak with EV-A71.[23] This was followed by Taiwan in 1998 reported 20 percent mortality in HFMD cases. In this outbreak, CV-A16 and EV-A71 types were found to be associated as etiological agents.[24] In Australia, during the years from 1991 to 2001 several HFMD cases were seen associated with CV-A16 as major etiological agent having neurological manifestations with involvement of central nervous system (CNS). Recently, HFMD reported in 2011 and 2014 from Croatia and Granada indicated CV-A16 as an etiological agent with neurological manifestations. Certain meteorological parameters like humidity and high temperature are found to be associated with HFMD susceptibility. In Asia, the temperate region HFMD is common during late spring and early summer.[24] In tropical and subtropical regions of Asia, outbreak occurs in the late spring, whereas other regions like Singapore, Thailand, Malaysia, and Vietnam, the association with humidity and temperature cannot be co-related, and therefore the outbreaks are reported throughout the year.[24]

In India, most of the HFMD cases were previously diagnosed earlier based on clinical features and symptomatic conditions. The first report on HFMD cases from India was documented from Calicut (Kerala) in the year 2005, where 81 cases were detected, and EV-A71 was identified as viral etiological agent.[25] Four cases of HFMD were further reported from Nagpur, Maharashtra.[5] In Jorhat, Assam, 34 cases of HFMD have been documented where the disease was diagnosed only on clinical features and symptomatic conditions.[26] Subsequently in the year 2009, cases of HFMD were reported from different parts of the country like Kerala, West Bengal, Orissa, and Tamil Nadu. Investigations conducted at ICMR-National Institute of Virology, Pune revealed the presence of CVA-16 infection (54.8%) followed by CVA-6, EV-A71, CVA-10, and Echo-9 [Figure 2].[27]

The study indicated CV-A16 as a major enterovirus type circulating in the reported regions. Cases of HFMD (n = 30) were reported in 2012 from Chennai with the involvement of CV-A16 as the viral etiological agent.[28,29] Genetic characterization of these strains of EVs identified in HFMD cases revealed emergence of CV-A16 B1c genotype, C1 genotype of EV-A71.[30] Along with CV-A16 and EV-A71, CV-A6 is also identified in sporadic cases in 2017–18 from Pune and Kolhapur regions of Maharashtra, Western India.[27] These detected CV-A16 strains exhibited 97–99% sequence identity with the Japanese and Chinese strains, whereas CV-A6 strains revealed 98–99% identity with the reported strains from Finland, Taiwan, and China.[31] These observations are in good concordance with the previous reports from India indicating CV-A16 and CV-A6 as major contributors among the other EVs causing HFMD.

Diagnosis

HFMD is usually diagnosed on the clinical basis but confirmation through molecular testing by using clinical specimens such as stool, vesicular swab (V.S.), throat swab (T.S.) etc., is further helpful in patient management. Many of the molecular procedures have been replaced by traditional methods of detection and characterization. First test is PCR, primarily used to detect EV genome in cell culture, clinical specimens, and biopsy/autopsy tissues. The second test is nucleic acid probe hybridization and is used for both detection and characterization, although usually with less sensitivity than PCR. The third and newest procedure utilizes genomic sequencing for the characterization of EV at the highest level of specificity.[32] The most important properties of these tests are that the primers are targeted to amplify the 5'UTR of the virus genome, since it is the most highly conserved region of genome among enteroviruses.[33] The other molecular methods involving dot blot hybridization and *in situ* hybridization with serotype-specific probes have been developed to detect the enteroviral genome, but these are less sensitive than PCR and are less widely used for detection of enteroviruses.[33] Nowadays, researchers are using real-time PCR-based assay for detection of enteroviral infections.

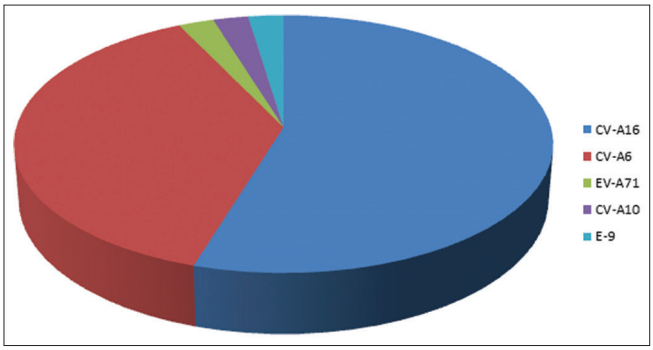

Figure 2: Identification of EV serotypes based on sequence analysis in India, 2012

Molecular Typing of Enteroviruses

Detection of enteroviruses is necessary to confirm and support the clinical diagnosis. However, molecular typing of enteroviruses is needed not only to define exact etiology of the infection but also to further classify enteroviruses, identify newer serotypes or variants and epidemiological monitoring of circulating serotypes. The molecular typing system is based on RT-PCR and nucleotide sequencing of the 3' half or the entire genome region encoding VP1. Molecular typing by sequence analysis, based on the close correlation of the serotype with the nucleotide sequence of the VP1 gene, is a new modality for enterovirus identification that has led to identification of several previously unknown serotypes if any, present in clinical/virus isolates. Detection of the virus in normally sterile sites (e.g., CSF, blood, pericardial fluid, tissue specimens) is considered and useful for diagnotic purpose. CV-A16 and EV-A71 virus can directly be detected in vesicular fluid collected from hands, feet, and buttocks of HFMD patients. Since enteroviruses are ubiquitous and because asymptomatic infections commonly occur, positive results from testing of non-sterile sites (e.g., stool samples, throat swab) should be interpreted with caution.

Virus Isolation

Virus isolation in cell culture is a standard technique applied for detection and characterization of enteroviruses. Isolation of enterovirus from different clinical specimens using specific cultured cell lines is often possible within 5 to 6 days. Stool specimens or rectal swabs, throat swabs or washings, and cerebrospinal fluid are the appropriate clinical specimens for virus isolation. No single cell line, however, exists that is capable of growing all human enteroviruses. Use of several different types of human and primate cells increases the spectrum of viruses that can be detected.

These cell lines include human rhabdomyosarcoma (RD) cell line Vero, Hep-2, HeLa, MDCK, CaCo-2, etc.[34,35]

Growth of enteroviruses in cell culture is identified by its cytopathic effect, i.e., the characteristic morphological changes such as visible rounding, shrinking, nuclear pyknosis, refractivity, and degeneration of infected cells.[32] However, virus culture is relatively insensitive, laborious, and time-consuming, and these factors limit the utility of virus culture.

Pathogenesis

The upper respiratory tract, oropharynx, and intestinal tract are the portals of entry for enteroviruses and are transmitted by the fecal–oral route. Viral replication is initiated in the mucosa and the lymphoid tissue of the tonsils and pharynx, and the virus later infects M cells and lymphocytes of the Payer's patches, and enterocytes in the intestinal mucosa. The virions are impervious to stomach acid, proteases, and bile. Primary viremia spreads the virus to receptor-bearing target tissues, including the reticuloendothelial cells of the lymph nodes, spinal cord, brain meninges, heart, spleen, and liver, to initiate a second phase of viral replication, resulting in a secondary viremia and symptoms. Virus can travel from the central nervous system via neural pathways to skeletal and cardiac muscles. The diseases produced by the enteroviruses are determined mainly by differences in the tissue tropism and the cytolytic capacity of the virus. Excretion of enterovirus by an infected individual starts at the end of the incubation period, and patients are most infectious shortly before and after onset of illness. Depending on the stage of the infection and the clinical syndrome, enteroviruses are found in oropharyngeal secretions, stool, cerebrospinal fluid, blood, and vesicular fluids.[36]

Earlier, there was no progress made in research on the pathogenesis of EV-A71 due to non-availability of suitable animal model. An experimental infection with EV-A71 in neonatal mice was reported. It was demonstrated that EV-A71 could infect one-day-old mice via oral inoculation. The pathogenesis study with EV-A71 strain was carried out in one-day-old ICR (Institute of Cancer Research, USA) mice. The mouse-adapted EV-A71 strain was used by oral inoculation route. The study revealed that mouse adaptation could increase the virulence of EV-A71 in mice.[37] MP4, a mouse-adapted EV-A71 strain with mutations in the VP2 and 2C regions and the 5' UTR showed increased virulence when it was delivered via oral inoculation. Studies on mutations in the mouse-adapted EV-A71 strain shed light on the neurovirulence of the virus. The study also shed light on the pathogenesis of CV-A16-induced disease.[38] Recently, pathogenesis studies were conducted in India using ICR neonatal mice for HFMD caused by CV-A16 virus, which revealed progressive pathological changes. These findings correlated with the symptomatic conditions initiated from day 3 onward of post-inoculation day (PID). The major affected ICR mice organs were found to be brain, heart, intestine, and skeletal muscles. In experiment, ICR mice strains caused minimal neuronal degeneration on day 3 of PID, and mild neuronal degeneration on day 7 of PID. A minimal enlargement of ventricles was observed in brain on day 3 of PID and was mild on day 7. Intestinal tissue revealed vacuolations in enterocytes which were minimal on day 3. Vacuolations in cardiomyocytes were seen on days 3 and 7 of PID. Mild degeneration in cardiomyocytes and infiltration of inflammatory cells were also observed in ICR mice strain on day 5 of PID.[39] A study conducted to investigate CV-A10 infection in rhesus macaques revealed that infection progresses through respiratory and digestive tracts causing herpes-like lesions on hand, feet, and in oral mucosa that are identical to these observed in humans causing HFMD. It also observed that CV-A10 viremia demonstrates that viral infection is dependent on its propagation and proliferation in the body. The process of virus assembly and proliferation can cause serious pathological damage to numerous organ tissues like heart and lungs resulting in pneumonia and myocarditis. It has also shown that inflammatory response elicited by CV-A10 not only damages tissues but also can lead to severe hyperglycemia after recovery from infection.[40]

Prevention and Treatment

Presently, there is no specific drug treatment available for enteroviral infections in clinical use. Interferon alpha and beta have been found to be effective in CV-A24 *in vitro* infections. Pooled immunoglobulin has been effectively used in enterovirus-induced CNS infection in immune-compromised patients.[2]

A number of specific antiviral compounds have been developed to target enteroviral proteins. The WIN compounds and related derivatives, which were originally shown to be effective against rhinovirus, have shown the most consistent results and have been those most studied mechanistically. These drugs bind a hydrophobic site near the surface of the virion called the pocket, which lies in the floor of the "canyon" where the virion binds to the cellular receptor. By binding to the pocket, these compounds are believed to interfere with viral uncoating and adsorption.[2]

Enviroxime, the antiviral drug that targets non-structural protein 3A, is leading to a block in the synthesis of plus-strand viral RNA. It is toxic and not effective in humans.[2]

In 1991, a new antiviral "pleconaril" was introduced, which is a novel orally bioavailable and systemically acting small molecule inhibitor for *Picornaviruses*. It is currently in clinical trials for treatment of enteroviral meningitis and respiratory infections.[11] In some of the cases, patient present with florid or unusual lesions with high febrile and

irritability. Such cases are considered as severe HFMD cases, and it is reported that oral acyclovir can be used as the promising drug.

It can be started in a dose of 10 mg/kg/dose for four times a day continued till day 7.[41]

CV-A16 vaccine-related studies have been carried out at Institut Pasteur of Shanghai, China, including the construction of an infectious cDNA clone of CV-A16.[42] Western blot protocol is established to quantify the yield of CV-A16 in Vero cells using recombinant protein VP0 as a standard reference and the development of inactivated and VLP CV-A16 vaccine.[37,43] Antisera from mice immunized with VLP antigen can neutralize CV-A16 virus in cell substrates and protect mice against lethal challenge. Anti-CV-A16 serum can neutralize homologous (CA16/SZ06) and heterologous (CA16/GX08) strains of CV-A16 in vitro. After 14 days homologous strains challenge of neonatal mice, the survival rates for groups receiving anti-VLP sera, anti-sf9 sera, and PBS were found to be 100%, 71.8%, and 44.4%, respectively, after 11-day challenge with a more virulent heterologous strain. These results indicate that neutralizing antibodies played important role in animal protection and lay the groundwork for development of CV-A16 VLP vaccines.

With regard to the development of inactivated vaccines, it has been demonstrated that anti-CV-A16-specific antibodies as well as T-cell IFN response can be induced in mice immunized with β-propiolactone-inactivated CV-A16 vaccines.

CV-A16, BJCA08 strain was used for the evaluation of the vaccine's efficacy in neonatal mouse model. Using a maternal antibody protection study and an antiserum protection study, it was demonstrated that the specific CV-A16 neutralizing antibody might block invasion of the virus and able to evaluate the protective efficacy of the CV-A16 vaccine.[37] It was reported that neonatal mice can be infected through I.P. inoculation and can led to severe clinical manifestations which demonstrates to establish productive infection in neonatal mice. Further it was documented that post-exposure treatment with anti-CV-A16 monoclonal antibody (mAb) 8C4 fully protected the mice against lethal CV-A16 infection.

The alternative and co-infection of CV-A16 and EV-A71 as well as the emergence of new recombination strains have made difficult to control the epidemic. Therefore, research studies were attempted on CV-A16 pathology, mechanism of pathogenesis, epidemiology and the development of CV-A16 vaccine, or EV-A71 and CV-A16 bivalent vaccine.[44,45]

Conclusions

HFMD is one of the diseases of concern which shows self-limiting to severe complications. At present, disease is cured by symptomatic treatment. So far in Indian context, it is found that majority of etiology is with CV-A16 and EV-A71, and also co-circulation of other EVs such as CV-A6, CV-A10, and Echo9 serotypes[46]. Molecular analysis of such clinical specimens is essential as genetic recombination may give rise to strains of high pathogenic potential. Early detection and confirmation using molecular typing will help patient management to reduce hospitalization, and prevent further spread of infection. In Indian scenario, there is a need to build up thorough surveillance system and detailed case-to-case studies to rule out the symptoms and severity of such notifiable disease. Currently, there is no surveillance system in our country to identify the disease as well as to know the circulating viral strains in different geographical regions. Such information will help further to plan the strategy for forthcoming challenges. Systematic HFMD surveillance is essential to know more epidemiological data. Studies conducted using ICR mice strains reveal progressive pathological changes and correlations with symptomatic conditions. Pathological studies indicate that brain, heart, and skeletal muscle are the potential target organs which suggest a wide spread of infection.

In view of the current scenario, presently many companies, organizations, and institutions in Southeast Asian countries especially China have begun to carry out research on CV-A16 vaccines. In early future, EV-A71 vaccine will be available in the market, and CV-A16 vaccine or bivalent vaccine (EV-A71 and CV-A16) is expected to be introduced in the clinical trials. Being headed for annihilation of polio virus, non-polio enteroviruses may cause great warning as HFMD with its several complications. Further, lack of effective therapy or vaccine is still anticipated and needs motivation on this aspect. These interventions will be supportive to control HFMD, which is a global public health concern.

Consent for publication

Yes.

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Conflicts of interest

There are no conflicts of interest.

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