



HAND, FOOT AND MOUTH DISEASE IN SOUTH BENGAL AND KOLKATA: A STUDY OVER 5 YEARS TO EVALUATE CASES WITH CLINICAL SUSPICION

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INTRODUCTION

Hand, foot, and mouth disease is a very infective infection. It's caused by viruses from the *Enterovirus* genus, among the *Enterovirus* genus coxsackievirus is most commonly found associated with Hand , Foot and Mouth disease. Hand, foot and mouth disease (HFMD) causes rashes or vesicular lesions in the affected individuals and lesions are found in extremities and upper extremity lesion is more common along with feet and mouth. It is mostly seen in school going children, and causative agents are likely Enterovirus-A (EV-A) species, including Coxsackievirus-A16 (CV-A16) and Enterovirus-71 (EV-71) [1]. Hand , Foot and Mouth Disease is usually mild and self-limiting. In the affected patient's first identified by a brief prodromal fever, followed by pharyngitis, mouth ulcers and rash on the hands and feet. The disease is caused by numerous members of the Enterovirus genus of the family Picornaviridae e.g. Coxsackievirus type A (CA) and Enterovirus 71 (EV71), and the clinical features are not identifiable and distinguishable from virus to virus. [2]. Young children have the highest risk of getting hand, foot, and mouth disease. Risk increases if they attend daycare or school, as viruses can spread quickly in these facilities. Children usually build up immunity to the disease after being exposed to the viruses that cause it. This is why the condition rarely affects people over age 10. However, it's still possible for older children and adults to get the infection, especially if they have weakened immune systems. EV71 is a human enterovirus A species causing infection in children[3,4]. Clinically though it is mild symptoms and self limiting initially, such as a fever along with unraised colorless spots, and bumps on the hands, feet, and mouth. In some patients with severe disease several neurological complications (including cephalomeningitis, encephalitis, and neurogenic pneumoedema) and circulatory disorders. Occasionally, it even causes death [5]. Therefore, an early indicator of EV71 infection with neurological involvement is crucial for appropriate management [6]. Hand, foot, and mouth disease by enterovirus infection reports severe complications (such as brain stem encephalitis, neurogenic pulmonary edema, and other fatal complications) and a high mortality due to HFMD are more frequently related to EV71 infection[7,8].

MATERIALS AND METHOD

Patients attending the dermatology outpatient department with typical clinical features of HFMD were included for the study after taking consent and filling consent form. All atypical cases were excluded. Cases were then selected for

virological analysis. Study was approved by Institutional Ethical Committee.

Cases of HFMD with classical presentation and having active oral lesions were selected during monsoon and postmonsoon session between July and November, in 5 successive years (2013, 2014, 2015, 2016 and 2017).

A total of 582 samples of throat swab were collected from children whose parents were agreed to include them for the study and gave consent. Throat swab samples were assessed for viral detection within 2–3 days on the onset of manifestation. All samples were collected in Viral Transport Medium transported -20 degree centigrade to the laboratory.

Viral RNA Extraction

Swab samples were suspended in 5 ml VTM in 15 ml sterile centrifuge tube (Hi Media). Supernatant was collected after centrifugation at 5000 rpm for 5 min at 4°C, and viral RNA was isolated using QIAamp viral RNA mini kit (Qiagen, Germany) as per manufacturer protocol from 140 µl supernatant. Viral RNA was eluted in 50 µl elution buffer and stored at –80°C for future use

Primers and Probes Used in The study are as follows

Table 1. The sequences of the primers and probes for CVA6 , CVA10, EV 71 , CVA 16

Type	Name	Nucleotide sequence (5'-3')
Primer	CVA6F	RCCGGATAGYAGRAAATCATAY
Primer	CVA6R	GGTGGATCRCTCAATTTWGC
Probe	CVA6P	FAM-TGGCAGACTGCTACTAACCCGT CGGTG-BHQ1
Primer	EV71F1	TTCATGTCACCYGCGAGYGC
Primer	EV71R1	GCYCCRTATTCAAGRTCTTTCTC
Probe	EV71P1	ROX-TAYGACGGRTAYCCCACRTTYGGW GA-BHQ1
Primer	CVA16F	CAAGTAYTACCTACRGCTGCCAA
Primer	CVA16R	CAACACACATCTMGTCCTAATGAG
Probe	CVA16P	CY5-TACCAGCACTRCAAGCYGCGGAG- BHQ1
Primer	IC F	GTCAAGATCCTCAAAGATACAGCT
Primer	ICR	ACTCTGGCCGTTGGTTTG
Probe	ICP	HEX-AGTTTGGAGTCTTGGATGTCGCAT- BHQ1

We used Taqman universal PCR Master mix (ABI , Thermofisher) , The samples were analysed by RTPCR by

following Protocol.

The reaction mixture contained 0.5 µmol forward primer, 0.5 µmol reverse primer, 1 µl (1–4 µg/µl), and 7 µl DNase-RNase free water added to the master mix (5 µl) kept ready in PCR tubes then 7 µl of Extracted RNA added to the tubes. CFX96 (BIORAD), was programmed as 50°C for 5 min initial Reverse Transcription, then Polymerase activation at 95°C for 20 second and amplification followed by 40 cycles of 95°C for 5 sec, 60°C for 30 sec, 72°C for 30 sec and 40°C for 5 min for the cooling step.

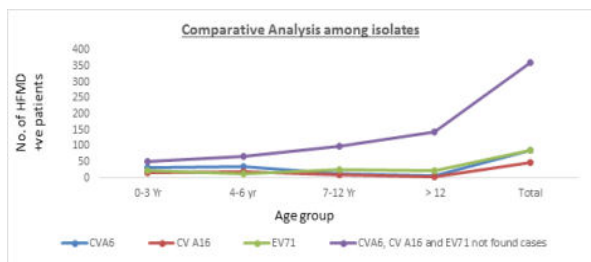
Transmission occurs from person to person through direct contact with saliva, faeces, vesicular fluid or respiratory droplets of an infected person and indirectly by contaminated articles. Usually, HFMD caused due to CV-A16 is less severe disease as compared to that caused by EV-71 [2]. However, severe complications, including deaths have been reported rarely [3]. In India, outbreaks of HFMD have been reported from various places, including Kerala, Odisha, Himachal Pradesh and Uttarakhand [4-7]. Furthermore, there has been no published report on epidemiological and clinical features of HFMD in the Andaman and Nicobar (A&N) Islands, a remote group of islands in the Bay of Bengal. In 2013, cases of HFMD were reported from various hospitals in Port Blair. The present study aimed to explore the epidemiology, clinical characteristics and causative agents of HFMD in these patients. These viruses can spread from person-to-person through direct contact with unwashed hands or surfaces contaminated with feces. It can also be transmitted through contact with an infected person's saliva, stool, or respiratory secretions.

Hand, foot, and mouth disease is characterized by blisters or sores in the mouth and a rash on the hands and feet. The infection can affect people of all ages, but it usually occurs in children under age 5. It is generally a mild condition that goes away on its own within several days.

RESULTS :-

Out of the 0-3 year age group Coxsackie Virus A 6 was found in 32 cases out of suspected 123 cases where as in the same age group Coxsackie Virus A 16 was found in 16 cases and Enterovirus 71 was found in 23 cases and 52 case none of these three virus families were found. In case of 4-6 year age group Coxsackie virus A 6 was found in 34 cases out of suspected 134 cases where as in the same age group Coxsackie Virus A 16 was found in 19 cases and Enterovirus 71 was found in 14 cases and 67 case none of these three virus families were found. In case of 7-12 year age group Coxsackie virus A 6 was found in 13 cases out of suspected 149 cases where as in the same age group Coxsackie Virus A 16 was found in 11 cases and Enterovirus 71 was found in 27 cases and 98 case none of these three virus families were found. In case of more than 12 year age group Coxsackie virus A 6 was found in 07 cases out of suspected 176 cases where as in the same age group Coxsackie Virus A 16 was found in 03 cases and Enterovirus 71 was found in 22 cases and 144 case none of these three virus families were found.

Table and Chart 1 :- Age wise different isolates of Hand , Foot and Mouth Disease



Age group	CVA6	CV A16	EV71	CVA6, CV A16 and EV71 not found cases
0-3 Yrs	32	16	23	52
4-6 yrs	34	19	14	67
7-12 Yrs	13	11	27	98
>12 Yrs	07	03	22	144
Total	86	49	86	361

Table and Chart 2 :- 0-3 Yrs Male Female different isolates of Hand , Foot and Mouth Disease

0-3 Year	CVA6	CV A16	EV71
Male	14	10	12
Female	18	6	11

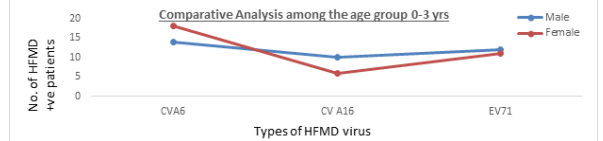


Table and Chart 3 :- 4-6 Yrs Male Female different isolates of Hand , Foot and Mouth Disease

4-6 Yr	CVA6	CVA16	EV 71
Male	13	09	06
Female	21	10	08

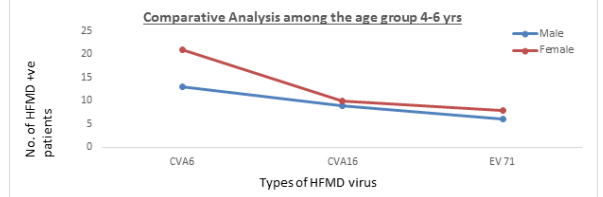


Table and Chart 4 :- 7-12 Yrs Male Female different isolates of Hand , Foot and Mouth Disease

7-12 Yr	CVA6	CVA16	EV 71
Male	06	04	08
Female	07	07	19

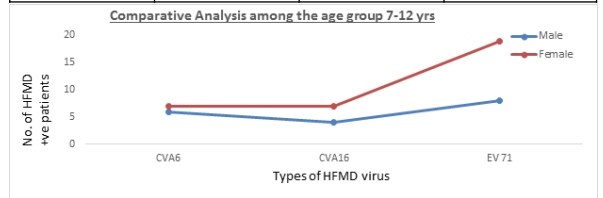


Table and Chart 5 :- more than 12 Yrs Male Female different isolates of Hand , Foot and Mouth Disease

> 12 Yr	CVA6	CVA16	Ev71
Male	02	03	06
Female	05	00	16

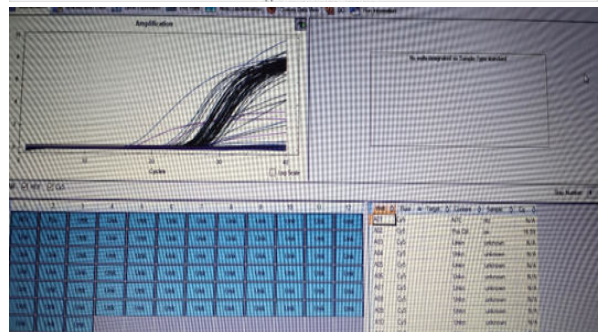
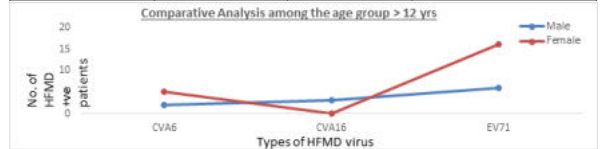


Image 1: RT-PCR Snapshot Taken From CFX96 Machine For Hand Foot And Mouth Disease

DISCUSSION

In our study we found most prevalent age group is 0-3 years followed by 4-6 years. Coxsackie Virus A 6 , Coxsackie Virus A 10 and Enterovirus 71 suspected cases are not belonging to these three Hand , Foot and Mouth disease causing virus types are maximum in more than 12 years old is maximum. 144 suspected cases are not belonging to these viral groups which can be attributed to other Coxsackie virus or Enterovirus genera or some Hypersensitivity reactions. Enterovirus 71 cases are more in more than 12 year old infants rather than below 12 year old babies or infants. Even Hand Foot and mouth Disease is found more in females rather than in male pediatric patients.

CONCLUSION

Various strains were isolated from suspected HFMD cases in India from recent years. Another recent publication in which participants and sampling were done from different regions of India reported the incidence of CVA16 (61.7%), CVA6 (34.04%), CVA4, and Echo12 (4.3%) among the 94 positive samples.[11] Another study from our country reported the incidence of CVA16 from Karnataka State almost half of the total cases (2 out of 4 cases).[12] CVA16 was also reported from Tribal population of Andaman island.[10]

Another multicentric study involved participants from many states including West Bengal, the state where this present study is being done, reportedly found CVA16 in all those cases.[9]. Our study however found the presence of Coxsackie Virus A 6, Enterovirus 71, Coxsackie Virus A 16 . Though our study included only these three viral types only. In future a study needed to be undertaken to study other rare viral types too.

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