

Occurrence of Enterovirus in hospitalised children with Acute Respiratory Infection at a tertiary health care center Jaipur

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Abstract

Background: *Enterovirus* (EV) has been reported to cause Acute Respiratory Infections (ARI) in infants and children. The aim of the present study was to detect the occurrence of EV in hospitalised children with ARI at Jaipur.

Materials and methods: Throat swabs and nasopharyngeal aspirates were collected from children hospitalised at J K Lone hospital. Extraction of viral nucleic acid was performed on an automated nucleic acid extraction system (Biomeuriex) as per manufacturer's instructions. 110 µl of viral nucleic acid was eluted from 400 µl of sample. Real-time RT-PCR was performed by using EV specific primer probe.

Results: Among the total 247 samples tested, 15(6.07%) children were found to be positive for EV. Males were predominantly infected with EV (66.67%) than females (33.33%). EV was predominant in the age group 1-12 months 14/200 (7.0%) and was associated with pneumonia in 33.33% and bronchiolitis in 13.33% cases among the positive samples. Circulation of the virus was observed in all the months of the study.

Conclusion: EV was detected in 6.07% of hospitalised children with ARI. Further studies are required in the state of Rajasthan to get a clear picture of EV epidemiology and its association in hospitalised children.

Keywords: *Enterovirus*, Acute Respiratory Infection, 1-12 months age group, real time RT-PCR

Introduction

Enteroviruses (EVs) are the members of the genus *Enterovirus* and the family Picornaviridae which are non-enveloped icosahedral particles measuring about 27–30 nm in diameter. The genome of the virus is a positive sense single stranded RNA molecule with 7400–7500 nucleotides in

length which encodes a large poly-protein [6]. EVs occur in respiratory secretions like the saliva, nasal mucus, sputum and stool. The virus is likely to spread from person to person when an infected person coughs or sneezes and infection can also occur by touching objects or surfaces having virus on them and then touching mouth, nose or eyes.

Hand sanitizers are ineffective against EVs [9].

EVs occur worldwide with major risk to young children, particularly males more than females. Most of the EV infections are subclinical in young children, but can also cause apparent disease and also clinical syndromes which involve different systems of the body. Upper Respiratory Tract Infections (URTIs) and summer colds are caused by non-polio EVs [9,1].

In U.S 10-15 million infections of EV occur every year. The spread of EV is not predictable, different types of EV can be common in different years with no pattern. Humans are the only natural hosts of EV [9]. Besides respiratory infections, EVs are also associated with other severe form of diseases like encephalitis, myocarditis, aseptic meningitis, hand-foot and mouth diseases [13]. The present study was undertaken to determine the occurrence of EV in hospitalised children of age ≤ 5 years with clinical features of respiratory infection.

Materials and methods

Calculation of samples size:

Sample size for the study was calculated as 163 by using the formula $n = 4 pq/l^2$ at an error rate of 4% with a prevalence rate of 7% for ARI in children under five years of age as reported by National Family Health Survey - 3.

Samples enrolled in the study:

A total of 247 samples from children of age ≤ 5 years were enrolled in the study with prior consent from the parent/guardian. The study was approved by the institutional ethics committee.

Clinical Samples:

Collection: Throat swabs and nasopharyngeal aspirates were collected from hospitalised children at J K Lone hospital (a paediatric tertiary health care centre at Jaipur) by a trained person as per

standard protocol of sample collection for the detection of respiratory viruses. Viral transport medium (VTM) was used for the collection of throat swabs and nasopharyngeal aspirates.

Transportation: Following sample collection, the samples were properly labelled and transported on ice at the earliest to Advance Basic Science and Clinical Research Laboratory, Department of Microbiology & Immunology, Sawai Man Singh Medical College, Jaipur.

Processing of Clinical samples:

The samples were processed as per established standard operation procedures (SOPs) of the laboratory for the detection of respiratory viruses.

Detection of HEV:

Extraction of viral nucleic acid was performed on an automated nucleic acid extraction system ((Biomeurieux) as per manufacturer's instructions. 110 μ l of viral nucleic acid was eluted from 400 μ l of sample. Following viral nucleic acid extraction, real-time RT-PCR was performed by using EV specific primer probe as described earlier [12] for the detection of EV.

Results

A total of 247 clinical samples from hospitalised children were tested for the presence of *Enterovirus*. These samples included 188 (76.11%) male children and 59 (23.89%) female children of age ≤ 5 years. Among the total samples tested 15(6.07%) children were found to be positive for *Enterovirus*. Males predominated with 10 (66.67%) than females with 5 (33.33%) among the positive samples.

EV in different Age-groups:

Large numbers of samples were enrolled in the age group 1-12 months as compared to rest of the age groups. The positivity of EV

was predominant in the age group 1-12 months 14/200 (7.0%) followed by 13-24 months age group 1/30 (3.33%) (Table-1). No positivity of EV was detected in other age groups.

Monthly activity of EV:

The circulation of the virus was observed in all the months of the study period from November 2013 - April 2014. EV was predominant in winter months of the study period and tends to decrease with the starting of the summer months. The activity of EV during different months is mentioned in (Table -1)

Association of EV with different clinical features:

Among the total EV positive samples the virus was associated with cough in 15/15(100%), fever in 13(86.67%), shortness of breath in 13 (86.67%) and pneumonia in 5(33.33%) of patients. EV was not detected in patients complaining of wheezing, sore throat and nasal catarrh (Figure-1).

Table -1: Age-wise and month-wise distribution of *Enterovirus* positive samples.

Year	Month	Age					Total samples
		1-12 months	13-24 months	25-36 months	37-48 months	49-60 months	
2013	November	3	-	-	-	-	3
	December	3	-	-	-	-	3
2014	January	3	-	-	-	-	3
	February	3	-	-	-	-	3
	March	1	1	-	-	-	2
	April	1	-	-	-	-	1
Total		14	1	0	0	0	15

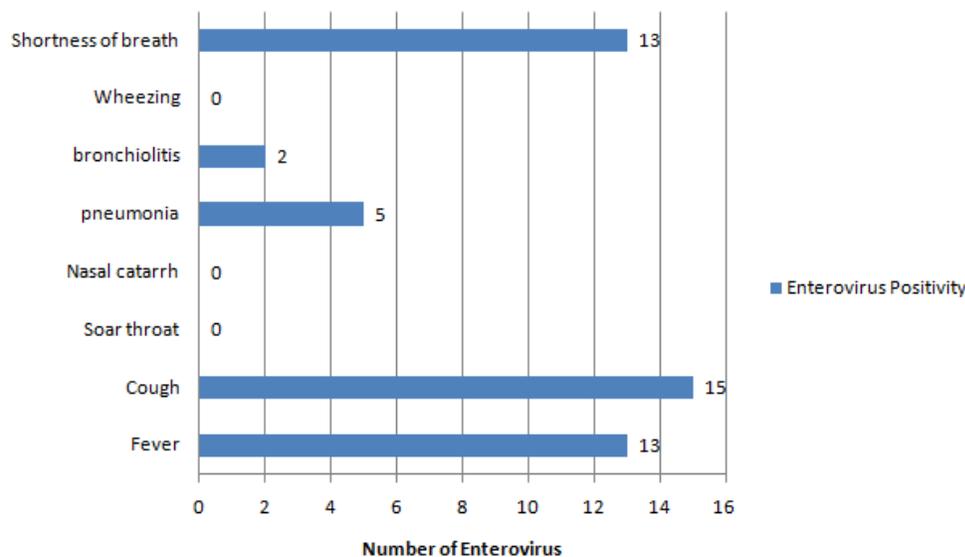


Figure 1: Clinical features observed in patients positive for EV.

Discussion

The present study reported EV in 6.07% of hospitalised children with ARI. This is consistent with an earlier study from Madagascar which reported a positivity of 5.70% in children with respiratory infection under 5 years [2]. EV was predominantly detected in male patients as compared to female patients in the present study. This finding is similar with an earlier EV surveillance study from Netherlands [8].

The present study reported EV in pneumonia (33.33%) and bronchiolitis (13.33%) of hospitalised children. These findings are in line with an earlier surveillance study from Netherlands on EV where hospitalisations occur every year due to EV with severe form of respiratory disease [8]. EV association with pneumonia and bronchiolitis was also reported by a previous study [10]. The present study reported a significant positivity of EV by real time RT-PCR. Loeffelholz and Chonmaitree reported RT-PCR to be a rapid and sensitive method for the detection of respiratory viruses [7]. However a multicentre study from Netherlands reported the sensitivity of RT-PCR depends on the reagents and the instrument used [3].

Our study reported a predominant positivity of EV in children up to 1 year of age as compared to children with higher age group. Previous study from Japan reported EV was detected predominantly in children with ARI of age group between 1-4 years [4]. In the present study EV was not detected in children of age group between >2 years to ≤ 5 years of age. The reason behind this may be due to low number of samples enrolled in this age group.

EV in our study was found to be predominant in the winter months and was found to be circulating throughout the study period. The peak activity was seen continuously from November 2013 - February 2014. Khetsuriani *et al.*, [5] reported EV in summer season and early

autumn in tropical climate. Whereas, study by Tokarz *et al.*, [11] reported EV in late summer months and winter months. However, EV may vary with its circulation patterns during different seasons in different geographical areas.

Conclusion

Enterovirus was detected in 6.07% of children with predominance in the age group of 1-12 months. EV was associated with pneumonia (33.33%) and bronchiolitis (13.33%) hospitalised children among the positive samples. Further studies are required in the state of Rajasthan to get a clear picture of EV epidemiology and its association in hospitalised children.

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Conflict of interest: None

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