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High Prevalence of Human Rhinovirus in Pneumonia Suspected Children of Uttar Pradesh Region, India

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A B S T R A C T

Childhood pneumonia is a common illness worldwide. Several bacteria and viruses can cause pneumonic illness among children. Respiratory viral infections are associated with childhood pneumonia up to 60% of the cases with high mortality rate. Human rhinoviruses (HRVs) associated pneumonia more likely to be occur in young children and older adults. The purpose of this study is to understand the prevalence of HRV in pneumonia suspected children of Uttar Pradesh region, using RT-PCR based molecular detection method. In this study 17.74 % children are found HRV positive. Children of 0 to 5 year age group are found more susceptible to infection with high HRV prevalence (68%). Clinical features of HRV confirmed pneumonic patients have been studied, among them 77% of patients were suffering from cough, followed by rhinitis (68.2%), sputum (63.6%), fever (59%), chest pain (54.5%) and shortness of breath (41%). Children showed clinical symptom of shortness of breath were below 5 years of age.

Introduction

WHO reported that acute respiratory infections are responsible for approximate 2 million death rate in paediatric population per year (Le *et al.*, 2012). In developing countries hospitalization, morbidity and mortality of children less than 5 years age group is mainly due to acute respiratory illness and pneumonia (Anh, *et al.*, 2011; Igor, *et al.*, 2008). The community acquired pneumonia is now a common problem in children caused either by several bacteria and viruses or their combination. Symptoms can include shortness of breath, coughing, fever, chills, chest pain and production of phlegm (Virkki *et al.*, 2002). Diagnostic techniques for differentiation of viral pneumonia from bacterial pneumonia are required for proper treatment of pneumonia and decreasing unnecessary use of antibiotics (Virkki *et al.*, 2002). Many viruses are responsible for serious respiratory illness such as influenza virus, respiratory syncitial virus (RSV), para

influenza virus, coronavirus, human meta pneumovirus and human rhinoviruses (HRVs) etc. **(**Ann *et al*., 2006; Dat 2013**).** HRVs, the causative agents of various upper and lower respiratory tract infections including common cold and pneumonia, are small, ss RNA viruses, these are the member of Picornaviridae family and currently divided into two species HRV A, HRV B and more than 100 serotypes have been found (Lourenço *et al*., 2014; Neil *et al*., 2007; Kathryn *et al*., 2007)**.** These HRVs as pathogen or co pathogen increase the severity of disease of respiratory infection mainly in case of bronchitis, bronchiolitis and pneumonia (Piotrowska Z. *et al*., 2009)**.** One study reported that in developing countries, HRVs association found with severe pneumonia both in pediatric and adult population and also in immuno suppressed subjects (Olli *et al*., 2013). According to another report, 24% rhinovirus is associated with childhood pneumonia (Virkki *et al*., 2002). In some cases, HRVs are responsible for life threatening pneumonia (Nikolaos *et al*., 2002). The association of HRVs in pediatric pneumonia has been studied previously but its association with viral pneumonia among pediatric population of North Indian region is still unknown. The aim of this study is to analyse the prevalence of HRV with clinical features in pneumonia suspected children of Uttar Pradesh region.

Materials and Methods

Sample Collection

124 throat and nasal swab samples of Pneumonia suspected children of 0-15 years age group were collected from King George Medical University, Lucknow, Uttar Pradesh, India.

Viral RNA Extraction

The viral RNA extraction of samples was done using the Viral RNA extraction Kit (QIAamp®, Qiagen) as per protocol provided with the kit.

Molecular Screening by PCR

5 µl of extracted viral nucleic acid was used for viral screening by the technique RT-PCR using one-step RT-PCR Kit (AgPath-ID, Life Technologies), as per the manufacturer's instructions. The forward primer 5'-GGGACCAACTACTTTGGGTG TCCG-3' and reverse primer 5'-CACGG ACACCCAAAGTAGT-3' (Kiang D *et al*., 2008) were used to amplify within the 5' Non transcriptional region (5' NTR).

During the single step PCR, the conditions used as follows- reverse transcription for cDNA synthesis at 50°C (30 minutes), Initial denaturation 95°C (10 minutes), followed by 40 cycles of denaturation at 95°C (30 seconds), annealing at 55°C (30 seconds) and elongation at 72°C (45 seconds).

Agarose Gel Electrophoresis

PCR products were loaded with gel loading dye and ethidium bromide on 1.5% agarose gel and run in TAE buffer. Products were visualized by use of ultraviolet illumination.

Results and Discussion

Here, we emphasized the involvement of human rhinoviruses in pneumonia among children of Uttar Pradesh region. In this study 124 throat and nasal swab samples of pneumonia suspected patients were examined by RT-PCR based molecular detection method using amplification of specific 5' Non transcriptional region (5'NTR) of viral RNA. 22 samples (17.74%) were confirmed as HRV positive by RT-PCR detection (figure-1), among them 63.6 % were male.

Table.1 Clinical Features of HRV Confirmed Pneumonic Children

Sample no.	Age	Sex	Cough	Sputum	Fever	Chest Pain	Rhinitis	Shortness of breath
5	3 year	Male	+	+	+	+	+	+
7	7 Year	Male	+	+	-	+	-	-
12	2 Year	Female	+	-	+	-	+	-
18	10 Year	Male	+	+	-	+	+	-
24	12 Year	Female	-	+	-	+	-	-
28	4 year	Male	+	+	+	+	+	-
42	9 month	Female	+	-	+	-	+	+
47	1 Year	Male	+	-	+	-	+	+
59	4 Year	Male	+	+	-	+	-	-
64	1 Year	Female	+	-	+	-	+	+
66	1.5 Year	Male	+	-	+	-	+	+
72	6 Year	Female	-	+	-	+	-	-
81	2 Year	Male	+	+	+	-	+	+
84	2 Year	Female	+	-	-	-	+	-
89	10 Year	Male	-	+	+	+	-	-
95	2.5 Year	Male	+	+	-	-	+	+
96	6 month	Male	-	-	+	-	+	-
102	3 Year	Female	+	+	+	+	+	-
109	11 Year	Male	-	+	-	+	+	-
111	7year	Female	+	+	-	+	-	-
116	1 Year	Male	+	-	+	-	+	+
119	5 Year	Male	+	+	+	+	-	+
			17 (77%)	**14** (63.6%)	**13**(59%)	**12**(54.5%)	**15**(68%	**9** (41%)

Fig.1 Gel Image Showing RT- PCR amplification of 5' NTR region of Human rhinovirus RNA. Lane M: Molecular marker1000 bp. Lane: 1, 6,7,9 PCR positive samples (400 bp).

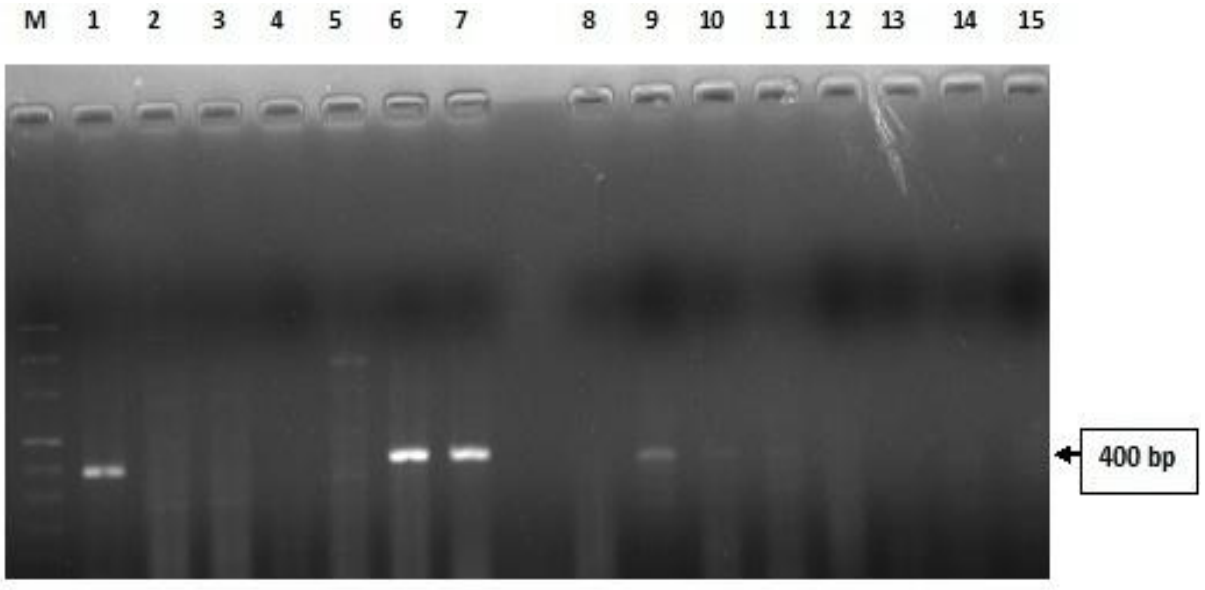
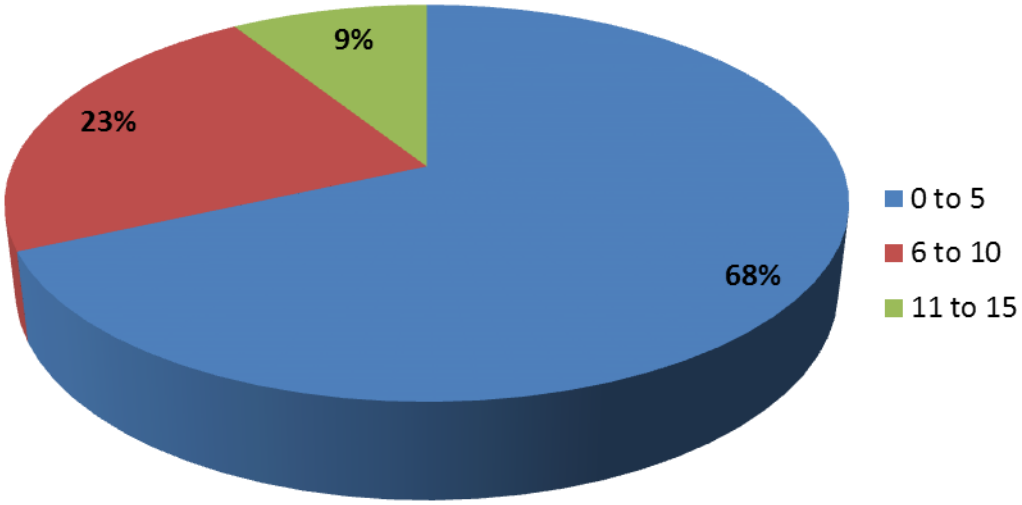


Fig.2 Percentage of HRV Positive Children with respect to age (years)



According to a previous study, In Italy 172 cases (29%) are found HRV positive among 592 children with pneumonia (Esposito *et al*., 2012). In a study on pediatric pneumonia (N=4279 episodes) using PCR methods, HRV infection found in 18% of the cases (Ruuskanen *et al*., 2011). Recent studies reported 11% to 53% cases of HRV associated pneumonia among the hospitalized children (Ruuskanen *et al*., 2011; Honkinen *et al*., 2012). Younger children of 0-5 years age group are found more susceptible for HRV infection. 68% of total HRV positive children were between 0-5 years of age. 23% children were 6 to 10 year old, while only 9% children were 11 to 15 year old (figure-2). Clinical features of HRV confirmed pneumonic patients have been studied. 77% of patients were suffering from cough, followed by rhinitis (68.2%), sputum (63.6%), fever (59%), chest pain (54.5%) and shortness of breath (41%) (Table-1). Children suffering from Shortness of breath were between 0 to 5 year age group that indicates the severity of disease among younger children.

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