Iranian Journal of Pediatric Society
Volume 2, Number 2, April-June 2010: 58-64

Original Article

Molecular Epidemiology of Respiratory Viral Pathogens in Children with Asthma Exacerbations Admitted to Dr. Masih Daneshvari Hospital

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ABSTRACT

Background and Aim: A mortality rate of four million children from Acute Respiratory Infections (ARI) is reported for developing countries. This study aimed to clarify viral etiologies, clinical manifestations, and other contributing factors (*e.g.* age, sex, seasonal distribution) of ARIs in referrals to Masih Daneshvari hospital, the research center for pediatric respiratory diseases in Iran.

Materials and Methods: Clinical samples, including nasopharyngeal swabs and nasal washings, were collected from 50 exacerbated asthma cases between October 2007 and September 2008. The specimens were collected from children aged less than 17 years old who were admitted to Masih Daneshvari Hospital, NRITLD, Tehran. Diagnosis was performed by nested or Real time PCR on specimens using DNA and RNA extracted with Invite Spin DNA and Invite Spin RNA Mini Kits, respectively.

Results: The 50 participating patients included 14 girls and 36 boys. The most common age group was 6-11 years, followed by 1-6 years. Cough and wheezing were the most commonly reported symptoms and signs, respectively. Rhinovirus was the most common causative agent. The most common season for infection was autumn, followed by winter, with a total rate of 78% under the category “cold season.”

Conclusion: Considering the small sample size, it would be advisable to perform a multidisciplinary survey over the country to obtain sufficient data to generalize the results and to help the health care system make suitable decisions regarding viral infection prevention and control, especially for respiratory tract infections.

Iranian J Pediatr Soc 2010; 2(2): 58-64

Keywords: *Asthma, Children, Viral respiratory infections*

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***Received:** February 2009; **Accepted:** December 2010*

INTRODUCTION

Acute respiratory infections (ARIs) are the major cause of hospitalization in infants and children, and viruses are typically the main infective agents (1-6). These microorganisms are also responsible for most of the global morbidity and mortality in that age group (7).

ARI is defined as the presence of rhinorrhea, cough, respiratory distress, and sore throat, with or without fever, which lasts maximally 7 days (8). Most respiratory viruses involve the upper respiratory tract (URT) and resemble the common cold with symptoms like coryza, cough, and hoarseness, while Lower Respiratory Tract (LRT) infections, which occur in one-third of cases, present with tachypnea, wheezing, severe cough, breathlessness, and respiratory distress (7).

Single strand RNA viruses, including *rhinovirus* (*RV*), *Influenza*, *Para influenza* (*PIV*), *Respiratory Syncytial* Virus (*RSV*), and *Corona* virus (*HCoV*), as well as DNA viruses like *adenovirus* (*AdV*), commonly lead to both upper and lower respiratory tract infections, among which *RSV*, *RV* and *influenza* virus could be named as the main causes (7,9). Recently identified viruses such as *human metapneumovirus* (*HMPV*), *human bocavirus* (*HBoV*), and *corona* virus (*HCoV*) are also potentially important respiratory pathogens (8).

Approximately 15-20% of children younger than 2 years of age experience RSV infection at least once (10). Typically, it presents as a mild upper respiratory tract infection (11), although it changes into serious cases when patients are in the first 6 months of life. In later years, asthma is a frequent consequence of long-term respiratory viral infections occurring before the age of 5 (7,10,11). Bronchiolitis is the other result of these viruses, which necessitates hospitalization of children (7,10) and imposes a high burden on families.

Host responses against viral particles are the predominant pathological aspects of respiratory tract infections and utilize both cellular and humoral arms of the immune system through inflammation process. This phenomenon can commonly be observed in *RV* and *HCoV* infections, as well as *RSV*-induced Upper Respiratory Tract Infections (URTI) (12,13), while *adenovirus* and *influenza* viruses promote conditions for cell lysis and tissue damage.

Lower Respiratory Tract Infection (LRTI) requires hospitalization in 1-2% of cases (7) and is worth examining to find at least a viral etiology, especially using viral antigen detecting techniques such as polymerase chain reaction (PCR) (7). In general, four methods are used for viral identification: virus culture and serology have been the gold standards for the past two decades, while immunofluorescent antigen detection and nucleic acid/PCR-based tests are coming to the fore. Culture and serology are more suitable for use in epidemiologic studies and/or patient follow-up because they take more time to generate appropriate results (7,14-16). In contrast, PCR is faster and significantly more sensitive and is poised to take over the field of virus diagnosis (7, 17-21).

The annual mortality rate due to ARIs for children in developing countries is estimated at four million (11). The aim of the present study was to clarify viral etiologies, clinical manifestations, and other contributing factors (*e.g.* gender, age, and seasonal differences) of ARIs in referrals to Masih Daneshvari hospital, the research center for pediatric respiratory diseases in Iran.

MATERIALS AND METHODS

Study design and participants: A descriptive cross-sectional design was used to enroll children under 17 years who were referred to our center between October 2007 and September 2008 because of LRTI. Complementary viral studies using PCR were conducted to determine the responsible viruses.

Participants were physically examined by a pediatrician to exclude suspicious viral cases. Chronic lung diseases, premature infants, low birth weight, neurological disorders, pulmonary or cardiac illnesses, and immunocompromised children were excluded because they were more prone to infections by *RSV* than by other pathogens (11,22,23).

In addition to viral types, this study also focused on patient age and sex and on the seasonal distribution of each type of virus on the one hand, and clinical manifestations, as well as their frequency and correlations to other virus types, on the other.

Clinical samples: Clinical samples, including nasopharyngeal swabs and nasal washings, were collected from 50 exacerbated asthma cases between October 2007 and September 2008. The specimens were collected from children aged less than 17 years, who were admitted to Masih Daneshvari Hospital, National Research Institute of Tuberculosis and Lung Disease, NRITLD, Tehran. The specimens were immediately transported in cold boxes (2-8°*C*) to the virology laboratory at the Virology Research Centre, NRITLD, and stored at -70°*C* until use.

Nucleic acid extraction and virus screening: DNA and RNA were extracted with Invite Spin DNA and Invite Spin RNA Mini Kits (Invitek, Germany), respectively. The presence of the *human bocavirus* (*hBoV*) genome was reported in our previous study, using nested Polymerase Chain Reaction (PCR) with primers in the NS-1 coding region (24).

Influenza A and B viruses, *respiratory syncytial virus* (*RSV*), *human metapneumovirus* (*hMPV*), *human coronaviruses* (*hCoV*), *parainfluenza viruses 1-3* (*PIV1-3*), *human rhinoviruses*, *human enteroviruses*, and *human adenoviruses* (*AdV*) were screened using real-time PCR, as described previously (25).

Statistics: The sample size was calculated as a group of 50 and the gathered data entered into SPSS V.12 for Windows to be analyzed using Chi-Square and Fisher's exact tests in order to extract descriptive findings. A 95% confidence interval and type one error of 0.05, along with p-value ≤ 0.05, were selected.

Ethics: Written informed consent forms were personally signed by the participants aged 15 years or more or by parents of children younger than 15, after being informed of the research aims and process.

RESULTS

The study participants consisted of 14 girls and 36 boys. The most common age group was 6-11, followed by 1-6 years.

Cough and wheezing were the most commonly reported symptoms and signs, respectively, as shown in table 1.

Although no significant correlation was found between fever and Post Nasal Drip (PND), 6 (17%) of 35 cases of fever had coincident PND. A similar association was seen between PND and cough. No noticeable relationship was found between the occurrence of pneumonia and the studied findings, even though fever was found in one-sixth of the cases. Dyspnea occurred in 4 out of 5 patients who had pneumonia (80%).

Table 2 summarizes the frequency of different leading microorganisms found following exacerbation of asthma in children entering the study. No statistically significant correlation was noted between the responsible microorganism and age groups except for *HBoV*, which occurred in 3 cases in the 12-36 month group (p= 0.031). The age distribution for pathogens was not

Table 1. Frequency of some symptoms and signs among the patients

Manifestations	Fever	Cough	Dyspnea	Rales	Wheezing	PND
No (%)	35 (70)	48 (96)	9 (18)	11 (22)	46 (92)	6 (12)

Table 2. The occurrence rate of each microorganism as the leading cause of infection

Pathogen	Bacterial	*RV*	*Enterovirus*	*RSV*	*InfA*	*InfB*	*HMPV*	*HPIV*	*AdV*	*HCoV*	*HBoV*
N (%)	7 (14)	11 (22)	1 (2)	2 (4)	1 (2)	0	0	3 (6)	4 (8)	6 (12)	3 (6)

Table 3. The most common involved age groups related to each pathogen

Pathogen	Bacterial	*RV*	*Enterovirus*	*RSV*	*InfA*	*HPIV*	*AdV*	*HCoV*	*HBoV*
Age group(mo)	12-36	12-36	73-132	73-132	73-132	36-72	73-132	36-72	12-36

significantly different, as shown in table 3.

Rhinovirus had the most frequent occurrence of fever (30%), but the relationship was not statistically significant. Dyspnea was found in 4 of 5 cases of *rhinovirus*, but again the correlation was not statistically significant. No correlation was found between enteroviral infection and the symptoms and signs of the disease. Dyspnea was the only statistically reportable finding among *RSV* cases (p=0.03) and was found in 2 out of 7 cases. Cough correlated with *parainfluenza* virus infection in this study (p=0.007), but no other finding showed the same relationship.

Table 4 shows the effects of seasonal distribution. No statistically significant correlation was noted between the pathogens and the season in which they occurred, except for *enterovirus*, which was reported only as one case that took place in the spring (p=0.027). The most common season for infections was autumn, followed by winter, with a total rate of 78% under the category "cold season."

DISCUSSION

A growing body of evidence now indicates that viral respiratory infections may be to blame for the pathogenesis of asthma and COPD (Chronic Obstructive Pulmonary Disease). Mild airway obstructions, along with hypersensitivities, can occur following episodes of these infections in children (26-33), adults (34,35), and animals (36-39), either naturally or in induced situations. A substantial proportion (30%) of children with pediatric asthma later develops adult asthma (40). The use of inactivated influenza vaccines (41,42), as well as current attempts to generate vaccines against *RSV* and *PIV* (9,43), now address the gravity of recognizing the viral etiologies responsible for respiratory infections through different periods of time in different geographical areas. PCR plays a central role in these studies.

Similar evidence has also been gained for symptoms, signs, patterns of complications, and distribution of viral respiratory infections. For example, Albuquerque (8) tested 205 cases of respiratory infected children between 4 months and 11 years and found a rate of 30.7% of viral etiology with a 16% frequency of *rhinovirus*. In the current study, the frequency of *rhinovirus* was 22%, with pneumonia and *HCoV* as the second and third most frequently found pathogens. In Albuquerque's work, *influenza-A* and *HMPV* followed *RV* in frequency. *Para influenza* was not reported in the previous study, but occurred at 6%

Table 4. Seasonal distribution of each pathogen

Pathogen	Bacterial	*RV*	*Enterovirus*	*RSV*	*InfA*	*HPIV*	*AdV*	*HCoV*	*HBoV*
Common season	Autumn-Winter	Autumn	Spring	Winter	Autumn	Autumn	Autumn	Autumn-Winter	Autumn
p-Value	0.25	0.96	0.027	0.192	1.00	0.30	0.68	0.47	1.00

frequency in our study. Albuquerque concluded that using nasopharyngeal swabs or aspirates could help to identify viral proportions for etiology.

Human bocavirus (*HBoV*), a new member of *Parvoviridae*, was found in 3 (6%) of our cases, while Karalar et al reported a 25% seroprevalence among children one year of age and less, and increasing to 93% after 3 years of age (44). This pathogen infects the LRT three times more frequently than the URT and has a rate 10% that of pneumonia.

Fé et al (45) studied three types of *HPIV* among nasopharyngeal aspirates from 3070 children. Of these, 933 were identified by screening to have respiratory viruses. The highest frequency was found for *HPIV*-type3 with an 83.76% rate and the lowest frequency was found for *HPIV*-type2. In the present study, the total rate of *HPIV* infection was 6%.

RSV, as the leading pathogen of bronchiolitis, was reported in 36% of Straliotto's cases in Brazil (46), whereas this virus was not common in our study (4%).

Cough (96%) and wheezing (92%) were the most predominant clinical manifestations, followed by fever (70%), in the present study. In contrast, Albuquerque et al named fever as the most common symptom, apart from etiologies of respiratory infection followed by rhinorrhea, cough, sore throat, and wheezing. Although no significant correlation was found between specific viruses and clinical findings in the current study, Straliotto reported a rate of crackles of 47.3% in *RSV* cases and exceeding 87% for *adenovirus*, but with no statistical significance. *RSV*-induced mortality rate was 2.8% in their survey and grew to 25% for *AdV*.

Seasonal distribution, as a significant aspect of epidemiology, was studied by Lee et al (23), who recruited 262 patients with acute bronchiolitis with a mean age of 11 months. They identified peaks of infection in both spring and autumn periods. Fé et al found *HPIV*-3 to be the only form of *HPIV* that showed a seasonal distribution, with a greater occurrence rate between September and November and an inverse correlation with rainy months.

CONCLUSION

In the present study, we determined autumn to be the most frequent season for the majority of cases, but no statistical significance could be reported. Enterovirus was diagnosed in just one case and infection occurred in spring. Co-infection was not evaluated in our study, although an up to 20% rate was reported for it by some authors (7).

Because of the small sample size used in the present study, a multidisciplinary survey should be performed over the country to obtain sufficient data to allow generalization of these results to the population. Confirmation of our findings could help the health care system to make suitable decisions for prevention and control of viral infections, especially in the respiratory tract.

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