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Molecular Study of *Respiratory Syncytial Virus*, Human *Rhinovirus* and Human *Metapneumovirus*, Detected in Children With Acute Wheezing

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ABSTRACT

Background: Viruses are known to cause the majority of acute respiratory infections. A great deal of evidence indicates that the etiology of most cases of wheezing in children, like asthma or bronchiolitis, is also linked to such respiratory infections.
Objectives: We assessed the prevalence of three common viruses including; *Respiratory syncytial virus* (RSV), human rhinovirus (HRV), and human *Metapneumovirus* (hMPV), in children with acute wheezing.
Patients and Methods: Ninety six wheezy children, 48 males (50%) and 48 females (50%) under the age of 5 years, were enrolled in the study. All patients visited as outpatients or inpatients when referred to the Mofid Children Hospital, in Tehran, from September 2009 to March 2010. A nasopharyngeal sample was taken from each child's nostril and the three viruses were detected by a molecular polymerase chain reaction method (PCR).
Results: Out of 96 patients, 63 cases (64.8%) had a positive PCR test for at least one virus. Prevalence of each virus including RSV, HRV and hMPV alone or in combination were 44 (45.8%), 13 (13.5%) and 6 (6.3%), respectively. There were no significant relationships between; age, prematurity, fever, respiratory distress and the existence of any kind of virus in the nasopharynx.
Conclusions: Our study revealed that the prevalence of these three viruses in the nasopharyngeal secretions of children suffering from acute wheezing was similar to other studies. The results of this study concluded; PCR assay is a widely available and rapid method to detect the viral etiology which induces wheezing in children in Iran, and the study also provides a baseline for future studies about the clinical importance of this relationship.

Implication for health policy/practice/research/medical education:
Since viruses are the main cause of asthma, especially in children under 5 years old, and they are also involved in the etiology of bronchiolitis, we decided to survey the prevalence of three main viruses; *Respiratory syncytial virus* (RSV), human *Rhinovirus* (HRV), and human *Metapneumovirus* (hMPV), in children with acute wheezing in our hospital. We hope that this study will provide a baseline for future studies about the clinical importance of particular viral prevalence.

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1. Background

Respiratory viruses are known to cause most acute respiratory infections (ARI) (1). The major viruses are; *Respiratory syncytial virus* (RSV), human *Rhinovirus* (HRV), *Parainfluenzae virus*, *Influenza virus*, *Adenovirus*, *Entrovirus,* and recently known viruses such as, human *Metapneumovirus* (hMPV), and human *bocavirus* (2, 3). Accumulated evidence indicates that the etiology of most cases of asthma, namely virus induced asthma, is linked to such respiratory infections (4, 5). Respiratory inflections by RSV, HRV and hMPV have been implicated in the induction of wheezing and the exacerbation of asthma (6).

Epidemiologic data suggests that about 70% of infants have experienced an RSV infection by the age of one year, and 100% by the age of two years (7). RSV is known to be a major causative agent of acute wheezing in children and may be strongly associated with asthma development later in life (8).

HRV was long believed to be the most frequently detected pathogen in the common cold, but recently it has been reported that there is also a higher prevalence of this virus in children with excacerbated asthma symptoms, than in those with well controlled disease (9, 10).

hMPV was first identified in 2001, and was reported to be one of the organisms of ARI in children (11, 12). Many epidemiologic studies have shown that, hMPV similar to RSV, is associated with bronchiolitis and recurrent wheezing in children (13-15).

2. Objectives

Epidemiological surveys on the prevalence of the causative viruses in children suffering from ARI and acute wheezing in Iran are limited. Therefore, we conducted our study to detect RSV, HRV and hMPV using polymerase chain reaction (PCR) on the nasopharyngeal secretions of children under 5 years of age, referred to one of the main children's hospitals in Tehran with acute wheezing illness.

3. Patients and Methods

A total of 96 children experiencing wheezing symptoms, under the age of five years, were enrolled in the present descriptive study. All of the children who were referred to the Mofid Children's Hospital with an ARI diagnosis and severe wheezing, during the cold months from September 2009 to March 2010, were seen as either outpatients or inpatients. Predefined exclusion criteria included; patients with acute wheezing and a diagnosis other than ARI, such as a foreign body aspiration or chronic disease like cystic fibrosis. Informed consent was obtained to take the nasopharyngeal swabs used in this study. The study protocol was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences.

For this study, two different diagnoses were classified as wheezing illness; bronchiolitis and bronchial asthma. Acute bronchiolitis was defined as; a first occurrence of expiratory wheezing with or without tachypnea, air trapping, and substernal retraction. Bronchial asthma was defined as recurrent wheezing $\geq$ two times in any child. Fever was defined as an axillary temperature $\geq 37.5^{\circ}$ C and respiratory distress, when children had tachypnea, with or without respiratory muscles retraction.

A sample of nasophaynx was taken from each child's nostril by gentle inserting a Dacron swab. The sample obtained was immediately put into a microtube containing 200 μL phosphate buffer saline (PBS) and sent to a pediatric research infection center (PIRC) laboratory, within 24 hours. The microtubes were stored at -70° C until reverse transcription-PCR (RT-PCR) processing for virus detection. All samples were thawed at 25° C, then the RNA virus was extracted using a high pure viral nucleic acid kit (Viral Gene-spin™ viral DNA/RNA kit, iNtRON Biotechnology, Inc, South Korea) and converted to cDNA using a special kit (Maxime™ RT-PCR PreMix, iNtRON Biotechnology, Inc, South Korea).

The extracted RNA was screened for RSV, HRV and hMPV by carrying out conventional PCR reactions. Specific primers for the nucleocapsid of these three viruses are shown in *Table 1*. Positive and negative controls were included in all of the PCR assays. PCR products were visualized under ultraviolet light after electrophoresis on a 2% agarose gel, stained with ethidium bromide. Data were analyzed using SPSS software (version 11.5), and P values < 0.05, were considered statistically significant.

Table 1. Prevalence of Three Viruses Alone or in Combination in the Nasopharyngeal of Patients

Viral PCR	No.	%
RSV	35	36.5
HRV	6	6.3
RSV + HRV	5	5.2
RSV + hMPV	4	4.2
HRV + hMPV	2	2.1
Negative PCR	33	34.3
Total	96	100

Abbreviations: hMPV, human metapneumovirus; HRV, human rhinovirus; RSV, respiratory syncytial virus

4. Results

We detected RSV, HRV and hMPV in the nasopharyngeal samples from 96 Iranian children, 48 males (50%) and 48 females (50%) with acute wheezing of whom 55 (57%) were referred as outpatients and the remaining 41 (43%) as inpatients. There were 33 (34%) patients who were diagnosed with acute bronchitis and 63 (67%) had a diagnosis of bronchial asthma. The prevalence of each virus including RSV, HRV and hMPV alone or in combination in the nasopharyngeal samples is shown in *Table 2*. Age distribution of the patients was; 59 patients (61.5%) under 1 year, 26 patients (27%) between 1 to 2 years, and 11 patients (11.5%) between 2 to 5 years.

Having a previous history of premature birth due to a high prevalence of RSV infection, was seen in 14 patients (14.6%), half of them gave a positive sample for RSV. Low grade fever (axillary temperature < 38.5° C) was seen in 58 patients (60.4%), moderate to high grade fever (axillary temperature ≥ 38.5° C) in 23 patients (24%) and the remaining 15 patients (15.6%) did not have a fever.

There were 42 patients (43.7%) who experienced respiratory distress and 54 patients (56.3%) that did not have any difficulty breathing. There were no significant relationships between the items above such as; age, prematurity, fever, respiratory distress and the existence of any kind of virus in the nasopharynx.

Table 2. Primers for PCR Used in This Study

Virus Primer	Sequence
RSV/forward	5-GGA ACA AGT TGT TGA GGT TTA TGA ATA TGC-3
RSV/reverse	5-CTT GAC TTT GST AAG AGC CAT TCT-3
HRV/forward	5-GCA CTT CTG TTT CCC C-3
HRV/reverse	5-CGG ACA CCC AAA GTA G-3
hMPV/sense primer L6	5-CAT GCC CAC TAT AAA AGG TCA G-3
hMPV/antisense primer L7	5- A CCC CAG TCT TTC TTG AAA-3

Abbreviations: hMPV, human metapneumovirus; HRV, human rhinovirus; RSV; respiratoy syncytial virus

5. Discussion

We detected three major viruses in the samples obtained from 96 Iranian children with acute wheezing during seven months; RSV, HRV and hMPV. These three viruses were detected in about two thirds of the patients (65.8%), with RSV being the dominant virus (45.8%). Many studies have suggested that RSV is a major candidate as an inducer of acute wheezing in children, and some of these children may go on to develop branchiolitis or pneumonia (16, 17).

Elhajja *et al*., demonstrated that RSV was the major etiology of bronchiolitis in children especially between 3-6 months and the combination of RSV and HRV was seen in 26% of the patients (18). Papadopoulos *et al*., demonstrated the presence of these viruses in 931 children under three years with acute wheezing; RSV was seen in 28.5%, HRV in 18.3% and hMPV in 6% of patients (19).

Although HRV has long been the most frequently detected pathogen in the common cold (9), Kotanimi-Syrjanen *et al.* were the first to recognize that HRV was also associated with early wheezing as a risk factor for asthma (20), other subsequent studies have confirm that HRV is an important risk factor for recurrent wheezing in children (5, 21). In contrast to RSV, the initial data suggests that HRV may be a potential determinant for the response to prednisolone treatment in wheezing children (22, 23). These findings emphasize the importance of searching for HRV in addition to RSV as a potential etiologic of bronchiolitis.

Several research groups have reported that hMPV is also an etiologic agent of wheezing illness in children (24, 25) and in a study by Garcia-Garcia *et al*., this virus was found to be the third most frequent virus after RSV and HVR in children under two years of age, with first incidence and recurrent wheezing (26). Moattari *et al*., in the only Iranian study, reported that in 120 children suffering from wheezing, hMPV was seen in about 20 patients (16.6%) (27). In spite of some previous studies that have emphasized that hMPV occurred predominantly in older children (28), in our study there was no significant age distribution between RSV and hMPV patients.

Although many other respiratory viruses can be detected by RT-PCR which can also induce wheezing in children, owing to budget restrictions, our study was limited to the detection of only: 1) RSV, the most common pathogen causing wheezing. 2) HRV, the virus that is probably the second most common etiology of wheezing induced by viruses. 3) hMPV, the novel virus that can be an important virus in the induction of wheezing.

We hope that with the advent of sensitive molecular testing, the clinical importance of pathogen detection becomes an increasingly practical method.For example is there any relationship between viral etiology of wheezy child and it's specific treatment?

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Authors' Contribution

None declared.

Financial Disclosure

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References

1. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev*. 2010;**23**(1):74-98.
2. Monto AS. Occurrence of respiratory virus: time, place and person. *Pediatr Infect Dis J*. 2004;**23**(1 Suppl):S58-64.
3. Fabbiani M, Terrosi C, Martorelli B, Valentini M, Bernini L, Cellesi C, *et al.* Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol*. 2009;**81**(4):750-6.
4. Pierangeli A, Gentile M, Di Marco P, Pagnotti P, Scagnolari C, Trombetti S, *et al.* Detection and typing by molecular techniques of respiratory viruses in children hospitalized for acute respiratory infection in Rome, Italy. *J Med Virol*. 2007;**79**(4):463-8.
5. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, *et al.* Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007;**119**(5):1105-10.
6. Busse WW, Lemanske RF, Jr., Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet*. 2010;**376**(9743):826-34.
7. Cane PA. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol*. 2001;**11**(2):103-16.
8. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med*. 2000;**161**(5):1501-7.
9. Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, *et al.* Rhinoviruses infect the lower airways. *J Infect Dis*. 2000;**181**(6):1875-84.
10. Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, *et al.* Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol*. 2007;**119**(2):314-21.
11. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001;**7**(6):719-24.
12. Howe M. Australian find suggests worldwide reach for metapneumovirus. *Lancet Infect Dis*. 2002;**2**(4):202.
13. Esper F, Boucher D, Weibel C, Martinello RA, Kahn JS. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. *Pediatrics*. 2003;**111**(6 Pt 1):1407-10.
14. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, *et al.* Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004;**350**(5):443-50.
15. Wilkesmann A, Schildgen O, Eis-Hubinger AM, Geikowski T, Glatzel T, Lentze MJ, *et al.* Human metapneumovirus infections cause similar symptoms and clinical severity as respiratory syncytial virus infections. *Eur J Pediatr*. 2006;**165**(7):467-75.
16. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, *et al.* The burden of respiratory syncytial virus infection in young children. *N Engl J Med*. 2009;**360**(6):588-98.
17. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, *et al.* Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med*. 2005;**171**(2):137-41.
18. El-Hajje MJ, Moulin F, de Suremain N, Marc E, Cosnes-Lambe C, Pons-Catalano C, *et al.* [Respiratory syncytial virus in hospitalized children. A 3-year study]. *Presse Med*. 2008;**37**(1 Pt 1):37-43.
19. Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, *et al.* Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med*. 2002;**165**(9):1285-9.
20. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003;**111**(1):66-71.
21. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, *et al.* Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008;**178**(7):667-72.
22. Jartti T, Lehtinen P, Vanto T, Hartiala J, Vuorinen T, Makela MJ, *et al.* Evaluation of the efficacy of prednisolone in early wheezing induced by rhinovirus or respiratory syncytial virus. *Pediatr Infect Dis J*. 2006;**25**(6):482-8.
23. Lehtinen P, Ruohola A, Vanto T, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol*. 2007;**119**(3):570-5.
24. Williams JV, Tollefson SJ, Heymann PW, Carper HT, Patrie J, Crowe JE. Human metapneumovirus infection in children hospitalized for wheezing. *J Allergy Clin Immunol*. 2005;**115**(6):1311-2.
25. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES. Detection of viruses identified recently in children with acute wheezing. *J Med Virol*. 2007;**79**(8):1238-43.
26. Garcia-Garcia ML, Calvo C, Perez-Brena P, De Cea JM, Acosta B, Casas I. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. *Pediatr Pulmonol*. 2006;**41**(9):863-71.
27. Moattari A, Aleyasin S, Arabpour M, Sadeghi S. Prevalence of human Metapneumovirus (hMPV) in children with wheezing in Shiraz-Iran. *Iran J Allergy Asthma Immunol*. 2010;**9**(4):250-4.
28. Kim YK, Kim JW, Wee YS, Yoo EG, Han MY. Clinical features of human metapneumovirus and respiratory syncytial virus infection in hospitalized children. *Pediatr Allergy Respir Dis*. 2009;**19**(1):12-9.