
Original Article

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

Soheila Khalilzadeh ¹, Mohammad Reza Boloorsaz ¹, Syed Ali Reza Nadji ², Syed Ali Reza Mahdavian ¹, Nooshin Baghaie ¹, Maryam Hassanzad ¹, Ali Akbar Velayati ¹

¹ Pediatric Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, ² Virology Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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

Corresponding author: Maryam Hassanzad, M.D.

Address: Pediatric Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Tel: +98-21-26109944; **Fax:** +98-21-26109944

E-mail: mar_hassanzad@yahoo.com

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 Stralioetto'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, Stralioetto 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.

REFERENCES

1. Glezen PW, Greenberg SB, Atmar RL, Piedra PA, Couch RB. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000; 283(4): 499-505.
2. Neuzil KM, Wright PF, Mitchel EF, Griffin MR. Burden of influenza illness in children with asthma and other chronic medical conditions. *J Pediatr* 2000;137(6): 856-864.
3. Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med* 2000; 342(4): 225-231.
4. Izurieta H, Thompson W, Kramarz P, Shay DK, Davis RL, DeStefano F, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med* 2000; 342(4): 232-239.
5. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations

- among US children, 1980-1996. *JAMA* 1999; 282(15): 1440-1446.
6. Counihan ME, Shay DK, Holman RC, Lowther SA, Anderson LJ. Human parainfluenza virus-associated hospitalizations among children less than five years of age in the United States. *Pediatr Infect Dis J* 2001; 20(7): 646-653.
 7. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev* 2010; 23(1): 74-98.
 8. Albuquerque MCM, Pena GPA, Varella RB, Gallucci G, Erdman D, Santos N. Novel respiratory viruses in children, Brazil. *Emerg Infect Dis* 2009; 15(5): 806-808.
 9. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001; 344: 1917-1928.
 10. Mohapatra SS, Boyapalle S. Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin Microbiol Rev* 2008; 21(3): 495-504.
 11. Palumbo E. Prevention of respiratory syncytial virus: a review. *Curr Respir Med Rev* 2009; 5(1): 55-58.
 12. Winther B, Gwaltney JM, Mygind N, Hendley JO. Viral-induced rhinitis. *Am J Rhinol* 1998; 12(1): 17-20.
 13. Hessel T, Baldwin CJ, O'Garra A, Openshaw PJ. CD8+ T cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection. *Eur J Immunol* 1997; 27(12): 3341-3349.
 14. Effler PV, Jeong MC, Tom T, Nakata M. 2002. Enhancing public health surveillance for influenza virus by incorporating newly available rapid diagnostic tests. *Emerg Infect Dis* 2002; 8(1): 23-28.
 15. Fan J, Henrickson KJ, Savatski LL. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription-polymerase chain reaction-enzyme hybridization assay (Hexaplex). *Clin Infect Dis* 1998; 26(6): 1397-1402.
 16. Storch GA. Rapid diagnostic tests for influenza. *Curr Opin Pediatr* 2003; 15(1): 77-84.
 17. Kehl SC, Henrickson KJ, Hua W, Fan J. Evaluation of the Hexaplex assay for detection of respiratory viruses in children. *J Clin Microbiol* 2001; 39(5): 1696-1701.
 18. Liolios L, Jenney A, Spelman D, Kotsimbos T, Catton M, Wesselingh S. Comparison of a multiplex reverse transcription-PCR-enzyme hybridization assay with conventional viral culture and immunofluorescence techniques for the detection of seven viral respiratory pathogens. *J Clin Microbiol* 2001; 39(8): 2779-2783.
 19. van Elden LJ, van Kraaij MG, Nijhuis M, Hendriksen KA, Dekker AW, Rozenberg-Arska M, et al. Polymerase chain reaction is more sensitive than viral culture and antigen testing for the detection of respiratory viruses in adults with hematological cancer and pneumonia. *Clin Infect Dis* 2002; 34(2): 177-183.
 20. van Milaan AJ, Sprenger MJ, Rothbarth PH, Brandenburg AH, Masurel N, Claas EC. Detection of respiratory syncytial virus by RNA-polymerase chain reaction and differentiation of subgroups with oligonucleotide probes. *J Med Virol* 1994; 44(1): 80-87.
 21. Weinberg A, Zamora MR, Li S, Torres F, Hodges TN. The value of polymerase chain reaction for the diagnosis of viral respiratory tract infections in lung transplant recipients. *J Clin Virol* 2002; 25(2): 171-175.
 22. Simon A, Ammann RA, Wilkesmann A, Eis-Hübinger AM, Schildgen O, Weimann E, Peltner, et al. Respiratory syncytial virus infection in 406 hospitalized premature infants: results from a prospective German multicentre database. *Eur J Pediatr* 2007; 166(12): 1273-1283.
 23. Lee JT, Chang LY, Wang LC, Kao CL, Shao PL, Lu CY, et al. Epidemiology of respiratory syncytial virus infection in northern Taiwan, 2001-2005: seasonality, clinical characteristics, and disease burden. *J Microbiol Immunol Infect* 2007; 40(4): 293-301.
 24. Nadji SA, Poos-Ashkan L, Khalilzadeh S, Baghaie N, Shiraghaei MJ, Hassanzad M, et al. Phylogenetic analysis of human bocavirus isolated from children with acute respiratory illnesses and gastroenteritis in Iran. *Scand J Infect Dis* 2010; 42(4): 598-603.
 25. Tiveljung-Lindell A, Rotzén-Ostlund M, Gupta S, Ullstrand R, Grillner L, Zwegberg-Wirgart B, et al. Development and implementation of a molecular diagnostic platform for daily rapid detection of 15 respiratory viruses. *J Med Virol* 2009; 81(1): 167-175.

26. Sly PD, Hibbert ME. Childhood asthma following hospitalization with acute viral bronchiolitis in infancy. *Pediatr Pulmonol* 1989; 7(3): 153-158.
27. Schroeckenstein DC, Busse WW. Viral bronchitis in childhood: relationship to asthma and obstructive lung disease. *Semin Respir Infect* 1988; 3(1): 40-48.
28. Nagayam Y, Sakurai N, Nakahara T, Makuta M, Honda A, Funabashi S, et al. 1987. Allergic predisposition among infants with bronchiolitis. *J Asthma* 1987; 24(1): 9-17.
29. Weiss ST, Tager IB, Muñoz A, Speizer FE. The relationship of respiratory infections in early childhood to the occurrence of increased levels of bronchial responsiveness and atopy. *Am Rev Respir Dis* 1985; 131(4): 573-578.
30. Glezen WP. Reactive airway disorders in children: role of respiratory virus infections. *Clin Chest Med* 1984; 5(4): 635-643.
31. Sherter CB, Polnitsky CA. The relationship of viral infections to subsequent asthma. *Clin Chest Med* 1981; 2(1):67-78.
32. McIntosh K. Bronchiolitis and asthma: possible common pathogenetic pathways. *J Allergy Clin Immunol* 1976; 57(6): 595-604.
33. Zweiman B, Schoenwetter WF, Pappano JE Jr, Tempest B, Hildreth EA. Patterns of allergic respiratory disease in children with a past history of bronchiolitis. *J Allergy Clin Immunol* 1971; 48(5): 283-289.
34. Laitinen LA, Elkin RB, Empey DW, Jacobs L, Mills J, Nadel JA. Bronchial hyperresponsiveness in normal subjects during attenuated influenza virus infection. *Am Rev Respir Dis* 1991;144(2): 1422-1423.
35. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976; 113(2): 131-139.
36. Piedimonte G, Nadel JA, Umeno E, McDonald DM. Sendai virus infection potentiates neurogenic inflammation in the rat trachea. *J Appl Physiol* 1990; 68(2): 754-760.
37. Dusser DJ, Jacoby DB, Djokic TD, Rubinstein I, Borson DB, Nadel JA. 1989. Virus induces airway hyperresponsiveness to tachykinins: role of neural endopeptidase. *J Appl Physiol* 1989;67(4):1504-1511.
38. Miura M, Inoue H, Ichinose M, Shimura S, Katsumata U, Kimura K, et al. Increase in lumina mast cell and epithelial damage may account for increased airway responsiveness after viral infection in dogs. *Am Rev Respir Dis* 1989;140(6):1738- 1744.
39. Massion PP, Funari CC, Ueki I, Ikeda S, McDonald DM, Nadel JA. Parainfluenza (Sendai) virus infects ciliated cells and secretory cells but not basal cells of rat tracheal epithelium. *Am J Respir Cell Mol Biol* 1993; 9(4):361-370.
40. Tabachnik E, Levison H. Infantile bronchial asthma. *J Allergy Clin Immunol* 1981; 67(5): 339-347.
41. Bridges CB, Fukuda K, Uyeki TM, Cox NJ, Singleton JA, Center for Disease Control and Prevention. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2002; 51(RR-3): 1-31.
42. Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P, et al. The efficacy of live attenuated, cold-adapted, trivalent intranasal influenza virus vaccine in children. *N Engl J Med* 1998; 338(2):1405-1412.
43. Murphy BR, Collins PL. Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. *J Clin Invest* 2002; 110(1): 21-27.
44. Karalar L, Lindner J, Schimanski S, Kertai M, Segerer H, Modrow S. Prevalence and clinical aspects of human bocavirus infection in children. *Clin Microbiol Infect* 2010; 16(6): 633-639.
45. Fé MM, Monteiro AJ, Moura FE. Parainfluenza virus infections in a tropical city: clinical and epidemiological aspects. *Braz J Infect Dis* 2008; 12(3): 192-197.
46. Straliotto SM, Siqueira MM, Machado V, Maia TM. Respiratory virus in the pediatric intensive care unit: prevalence and clinical aspects. *Mem Inst Oswaldo Cruz* 2004; 99(8): 883-887.