Viral etiology of acute lower respiratory tract infections in hospitalized young children in a children's referral hospital in Iran

Babak Pourakbari[1], Shima Mahmoudi[1], Zahra Movahedi[2], Shahnaz Halimi[3], Shervin Momeni[4], Reihaneh Hosseinpour-Sadeghi[1], Setareh Mamishi[1,4]

[1]Pediatric Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, [2]Department of Infectious Disease, School of Medicine, Qom University of Medical Sciences, Qom,[3]Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran,[4]Department of Infectious Disease, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail:smamishi@sina.tums.ac.ir

SUMMARY: Pourakbari B, Mahmoudi S, Movahedi Z, Halimi S, Momeni S, Hosseinpour-Sadeghi R, Mamishi S. Viral etiology of acute lower respiratory tract infections in hospitalized young children in a children's referral hospital in Iran. Turk J Pediatr 2014; 56: 354-359.

Viruses are considered major causes of acute respiratory tract infections among children under 5 years old. In this study we investigated the prevalence of three respiratory viruses—respiratory syncytial virus (RSV), influenza virus (INF) and adenovirus (ADV)—among hospitalized children with acute viral lower respiratory tract infections (LRTIs). Nasopharyngeal aspirates were collected from children under five who had been hospitalized for LRTIs. The clinical data, including demographic data (age and sex), vital symptoms and signs at admission, duration of fever, duration of hospitalization, chest X-ray findings and outcome were considered.

All inpatient specimens were tested by reverse transcriptase-polymerase chain reaction (RT-PCR) for RSV and the INF-A, INF-B and parainfluenza viruses and by polymerase chain reaction (PCR) for ADV. Out of those from 232 patients, 58 (25%) specimens were positive for either RSV, INF or ADV. The most predominant pathogens were RSV (40 cases, 17.2%), followed by INF (10 cases, 4%; including 8 type A and 2 type B) and ADV (8 cases, 3.4%). A total of 32 (55.1%) viral cases were identified in the spring, followed by 19 (32.7%) in the autumn and 7 (12%) in the winter. There was no significant correlation between clinical symptoms and the individual virus detected. In our study, RSV and INF were the two most common causes of LRTIs. These data are helpful for guiding the development of further vaccines as well as the use of antiviral drugs. Further studies will be needed to investigate other respiratory viruses such as parainfluenza, human metapneumovirus and rhinovirus.

Key words: respiratory viruses, children, Iran

According to data from the World Health Organization (WHO), acute respiratory infections (ARIs) are responsible for at least six percent of the world's disability and death[3].

Hospitalization ARIs in young children pose a substantial burden on health services, particularly in developing countries. Throughout the world, 1.9, 3.9 and 1.5 million deaths due to acute respiratory infection were reported by WHO in 2000, 2001 and 2008 respectively[2,4].

Respiratory viruses are the second most common cause of mortality and morbidity in children under 5[1,2]. The clinical manifestations and epidemiological features of respiratory virus infection vary in different geographic areas[5].

Various types of viruses were considered as important causes of acute respiratory infection around the world[6-8]. Respiratory syncytial virus (RSV), influenza virus (INF), adenovirus (ADV) and parainfluenza virus (PIV) are considered as the leading causes of acute viral lower respiratory tract infections (LRTIs)[9,10]. The

etiologies of LRTIs in children differ according to the age of the child, the season, the clinical presentation, the population studied, the case definition, the clinical specimens and the test methodology. This study was performed to identify the epidemiology of viruses causing ARIs among children under 5 years of age hospitalized at the children's medical center in Tehran, Iran, using reverse transcriptase-polymerase chain reaction (RT-PCR) testing for RSV, INF-A, INF-B and PIV and polymerase chain reaction (PCR) testing for ADV.

Material and Methods

Patients and respiratory specimens

During January to December 2012, 232 children (2-60 months of age) with respiratory infections were studied in the children's referral hospital, a tertiary medical facility located in Tehran, Iran.

Eligible children were under 5 years of age and had received a diagnosis of LRTI (pneumonia or bronchiolitis), which was defined as an illness presenting with one or more of the following symptoms. Acute respiratory illness was defined by the presence of rhinorrhea, cough, sore throat and/or tachypnea, either in association with or in the absence of fever with a maximal duration of 7 days. All of the patients were healthy before admission. They were hospitalized due to the severity of their illness, or their intolerance of oral feeding. The illness in question in all patients was the first severe LRTI leading to hospitalization. This study was approved by the Ethics Review Committee of the Tehran University of Medical Sciences, and written informed consent was obtained from a parent or guardian of each child.

Nasopharyngeal aspirates were collected through a feeding tube within 48 hours of admission. Samples were taken by experienced nurses on the infectious ward. Collected samples were stored in a freezer at -70˚C for subsequent DNA or RNA extraction.

Viral diagnosis

All inpatient specimens were tested by RT-PCR for RSV, INF-A, INF-B and PIV and by PCR for ADV.

Viral nucleic acid (DNA/RNA) was extracted simultaneously from 200 μl of patient nasopharyngeal aspirate using a QIAamp MinElute Virus Spin Kit and QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Total RNA extracted from the clinical samples was used for the synthesis of the first-strand cDNA. With the exception of human adenovirus, the respiratory viruses tested for in this study were RNA viruses; therefore, the conversion of RNA into cDNA was essential in order to be amplified using conventional PCR methods.

In this study, cDNA synthesis was performed using random hexamers and a RevertAid™ M-MuLV (molony-murine leukemia virus) enzyme for the reverse transcription process (Fermentas, Thermo Fisher Scientific Inc.). Reverse transcription was performed for 1.5 h at 37°C in a final reaction volume of 20 μl with 1 μl of random hexamer, 8 μl of total RNA, 1 μl of reverse transcriptase, 1 μl of RNase inhibitor, 2 μl of dNTP, 4 μl of 5× RT buffer and 3 μl of water. The products were stored at -20°C until used.

All specimens were tested for respiratory viruses using reverse transcriptase-polymerase chain reaction (RT-PCR), with forward:5'- GAC CRA TCC TGT CAC CTC TGA C -3' and reverse: 5'- AGG GCA TTY TGG ACA AAK CGT CTA -3' primers for INF-A , forward primer 5'- TCC TCA ACT CAC TCT TCG AGC G -3' and reverse primer 5'- CGG TGC TCT TGA CCA AAT TGG -3' for INF-B, and forward primer 5'- GGC AAA TAT GGA AAC ATA CGT GAA -3' and reverse primer 5'- TCT TTT TCT AGG ACA TTG TAY TGA ACA G -3' for RSV. In addition, PCR with primers F: 5'- GCC CCA GTG GTC TTA CAT GCA CAT C -3' and R: 5'- GCC ACG GTG GGG TTT CTA AAC TT -3' was performed for ADV(11).

Detection of the viruses was carried out with the following components in a 25 μl reaction volume: 1 μl of DNA, 10 pmol of the specific primers, 0.3μl of Taq DNA polymerase (0.5U/μl) (Invitrogen), 1 μl of $MgCl_2$ (50 mM) (Invitrogen), 2.5 μl of buffer (10x) (Invitrogen), 2.5 μl of dNTPs (2 mM).

PCR amplification conditions were as follows: initial denaturation step at 95°C for 15 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30s (for RSV, INF-B and ADV) or 52°C (for INF-A), extension at 72°C for 2 min, followed by a final extension step

at 72°C for 10 min.

To compare the distribution of the various respiratory viruses during different seasons, the period from January to December 2012 was considered.

Clinical data

The principal investigator determined the clinical diagnosis on the basis of a review of the patient's medical record. The clinical data, including demographic data (age and sex), vital symptoms and signs at admission, duration of fever, duration of hospitalization, chest X-ray findings and outcome were entered into a database by an individual who did not have knowledge of the viral identities.

Statistical analysis

All data were expressed as mean± standard deviation or median with range. Categorical data were analyzed using a Fisher Exact test, considering a p value <0.05 as significant. Statistical calculations were performed using the SPSS 18.0.1 statistical package (SPSS Inc, Chicago).

Results

During the surveillance period, a total of 232 specimens were collected. Patients' ages ranged from 6 to 60 months (median 17.3 ± 13.3).

Fifty-eight (25%) respiratory viruses were detected. The most predominant pathogen was RSV (40 cases, 17.2%), followed by INF (10 cases, 4%; including 8 type A and 2 type B) and ADV (8 cases, 3.4%). We did not detect co-infection with multiple viruses in our patients.

Age and Gender Distribution

Among the patients, 119 (51.3%) were male and 113 (48.7%) were female. Twenty-seven (67.5%) of the RSV-infected children were boys and 13 (32.5%) were girls. In the influenza and adenovirus groups, 7 (70%) and 6 (75%) patients were male and 3 (30%) and 2 (25%) were female, respectively. The mean age of influenza-infected patients was 27.9± 9.6 months, as compared to 18.6± 12.6 months for those infected with RSV and 21.2 ± 14.6 months for those infected with adenovirus.

LRTIs caused by RSV were predominant among younger infants (18.6± 12.6 months), as compared to LRTIs associated with influenza (27.9± 9.6 months) and adenovirus (21.2 ± 14.6 months).

Seasonal Distribution

The seasonal distribution of positive samples is shown in Fig. 1.

The results of this study showed that the prevalence of viral respiratory infections was highest in the spring, followed by autumn and then winter.

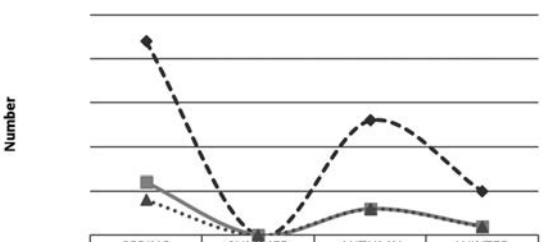
The presence of RSV infections was detected year-round except in the summer, with a peak occurring during the spring.

Distribution of Clinical presentation

The clinical diagnoses of the patients with LRTIs associated with these 3 common viruses are summarized in Table I. The three most prevalent symptoms were tachypnea, chest retraction and wheezing. Comparing the clinical data between children with RSV, INF and ADV infections, most variables showed no significant difference. Among all RSV-positive inpatients, 75% had tachypnea, 57.5% had rhinorrhea and 55% had wheezing. Tachypnea was associated more with RSV than with the other viral respiratory infections, while cases of INF infection were more likely to be associated with wheezing and cyanosis than were the other types of infection (P value≥ 0.5).

We did not have any mortality due to LRTIs during our study. All children with LRTIs had no coexisting medical conditions or characteristics that significantly identified them as being at greater risk for acquiring such an infection.

Among the 58 cases with RSV, ADV or INF, 21 patients (36.2%) had an abnormal chest X-ray (Table II; some patients had more than



	SPRING	SUMMER	AUTUMN	WINTER
RSV	22	0	13	5
INF	6	0	3	1
ADV	4	0	3	1

Fig. 1. Seasonal distribution of respiratory viral agents (ADV, RSV and INF)

Table I. Demographic Data and Clinical Presentation of Children with Acute Lower Respiratory Tract Infections

Clinical presentation		RSV (n = 40)	INF (n = 10)	ADV (n = 8)
Sex	Male	27 (67.5%)	7 (70%)	6 (75%)
	Female	13 (32.5%)	3 (30%)	2 (25%)
Duration of hospitalization (days)		5 ± 0.4	4 ± 0.6	4 ± 0.7
Duration of symptoms (days)		4.7 ± 1.8	5.1 ± 1.4	4.3 ± 1.3
Mean temperature (˚C)		36.9 ± 1.8	37.5 ± 0.6	37.9 ± 0.9
Pharyngitis		9 (22.5%)	3 (30%)	2 (25%)
Conjunctivitis		10 (25%)	4 (40%)	3 (62.5%)
Severe pneumonia		17 (42.5%)	3 (30%)	2 (25%)
Rhinorrhea		23 (57.5%)	6 (60%)	4 (50%)
Wheezing		22 (55%)	7 (70%)	5 (62.5%)
Cyanosis		20 (50%)	6 (60%)	4 (50%)
Tachypnea		30 (75%)	6 (60%)	5 (62.5%)
Chest retraction		26 (65%)	6 (60%)	5 (62.5%)
Abnormal chest X-ray		15 (37.5%)	4 (40%)	2 (25%)

RSV: respiratory syncytial virus, INF: influenza virus

one abnormal finding). The chest radiograph confirmed the diagnosis of pneumonia and indicated a complication such as a pleural effusion, empyema, hyperinflation with bilateral interstitial infiltrates or peribronchial cuffing. In 3 patients, *Streptococcus pneumonia* was isolated from the blood culture. In patients with signs and symptoms of bacterial infection, such as lobar consolidation, air bronchogram on the chest X-ray, plural effusion or empyema, appropriate antimicrobial agents were started.

Discussion

In this study, we evaluated the overall prevalence of the most frequently found respiratory viruses, identified by the RT-PCR and PCR methods, among children with LRTIs.

In our study, similarly to other investigations[12-14], RSV was found to be the predominant virus among hospitalized children under five years old with LRTIs. However, this result was in contrast to results reported from Taiwan, Brazil and Vietnam[15-17]. In our study, 40 cases (17%) were positive for RSV. This was similar to findings in Iran (19.4%)[18], Malaysia (18.4%)[19] and the United States[20]; a study conducted in Turkey showed a higher frequency (32%)[21].

Influenza was the second most common virus, a finding that was consistent with the study by Broor et al. in India[22]. In Egypt, RSV and ADV were shown as the second most common viruses in patients under 6 months and 7-12 months old, respectively[23].

The prevalence of ADV in our patients was 3.4%, which was lower than that reported in other studies[21,24,25].

In our investigation, the majority of viral respiratory infections were found to occur in early spring, autumn and winter, in that order. It might be due to the weather in our country, which in early spring is still cold.

In the study by Xie et al.[13], the epidemic seasons for RSV were winter and spring. In other studies, conducted in India and Italy, the most cases with RSV infections were seen in fall and winter[22,26], but the peak of adenovirus infection was in April[13,23].

Although most of the cases with confirmed respiratory viruses occurred in patients under 1 year of age, there was no significant association between age and individual viruses in our patients. However, there are some limitations,

Table II. Chest X-ray Findings of 21 Patients with Abnormal Findings

	N	%
Lobar consolidation	3	14
Air bronchogram	5	24
Hyperinflation	15	71
Interstitial infiltration	18	86
Pleural effusion	1	5

due to selection of patients under 5 years old.

In some other investigations, such as the study by Lina et al[24]., which was conducted using broader age groups, the prevalence of ADV was more frequent in children 1 to 4 years old.[24]

The frequency rates of all viruses isolated in our patients were higher in boys (68.9%). The higher susceptibility of boys that has been reported in several studies might be due to the smaller diameter of the airways in boys[9,27,28].

In the present study, no significant difference was detected in the clinical signs and symptoms related to individual viruses. In some studies, a greater severity of symptoms has been reported in RSV infection than in that caused by other viruses[24]. In our study, 37.5% of patients had a normal temperature. The mean temperature was 37.6 ± 0.8. This was lower than in other studies, especially in patients with adenovirus infections, which are usually associated with high fever[24]. Possible explanations for this result may be administration of antipyretic agents by the parents or poor accuracy in temperature determination. Cyanosis was detected in nearly half of the cases, which was a finding similar to that of studies conducted in Taiwan and Korea[16,29]. In our study 70% of patients presented with tachypnea, a rate similar to that reported in studies from France and Korea[12,26].

We did not detect co-infections with multiple viruses in our patients, whereas in the study by Albuquerque et al.[15] in Brazil and that by Fabbiani et al.[26] in Italy there were, respectively, 8 (3.9%) and 18 (16.5%) cases in which co-infection with more than one virus was reported.

The rate of viral infection in our patients was 25%, but this rate would in all probability be higher if we tested for more respiratory viruses. Definition of the viral agents in respiratory tract infections helps to identify the burden of viruses and prevent immethodical antibiotic administration.

Conclusion

RSV, INF and ADV were the predominant causes of viral respiratory infections in children under 5 years old. The prevalence of respiratory viral infections was highest in spring, followed by autumn and winter. Accurate and timely diagnosis of the cause of LRTI in children can reduce unnecessary antibiotic use and decrease the overall cost of care. In addition it has potential benefits, including the development of new vaccines and the administration of antiviral drugs for the prevention and treatment of viral respiratory tract infections. Because we tested for only three common viral agents, further investigation will be needed to detect other respiratory viruses such as PIV, human metapneumovirus and rhinovirus.

REFERENCES

1. Denny FW, Loda FA. Acute respiratory infections are the leading cause of death in children in developing countries. Am J Trop Med Hyg 1986; 35: 1-2.
2. Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis 2002; 2: 25-32.
3. Huang G, Yu D, Mao N, et al. Viral etiology of acute respiratory infection in Gansu province, China, 2011. PLoS One 2013; 8: e64254.
4. Statistical Annex Table 3: Burden of disease in DALYs by cause, sex and mortality stratum in WHO regions, estimates for 2001. In: The World Health Report 2002. Geneva: World Health Organization; 2002: 192-197.
5. Choi EH, Lee HJ, Kim SJ, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clin Infect Dis 2006; 43: 585-592.
6. Bourgeois FT, Valim C, Wei JC, McAdam AJ, Mandl KD. Influenza and other respiratory virus–related emergency department visits among young children. Pediatrics 2006; 118: e1-e8.
7. Shek LP, Lee BW. Epidemiology and seasonality of respiratory tract virus infections in the tropics. Paediatr Respir Rev 2003; 4: 105-111.
8. Tůmová B, Heinz F, Syrůcek L, et al. Occurrence and aetiology of acute respiratory diseases: results of a longterm surveillance programme. Acta Virol 1989; 33: 50-62.
9. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatrics 2004; 113: 1758-1764.
10. Meerhoff TJ, Mosnier A, Schellevis F, Paget WJ. Progress in the surveillance of respiratory syncytial virus (RSV) in Europe: 2001-2008. Euro Surveill 2009; 14: 19346.
11. Kim C, Ahmed JA, Eidex RB, et al. Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One 2011; 6: e21610.
12. Kwofie TB, Anane YA, Nkrumah B, Annan A, Nguah SB, Owusu M. Respiratory viruses in children hospitalized for acute lower respiratory tract infection in Ghana. Virol J 2012; 9: 78.

13. Xie ZD, Xiao Y, Liu CY, et al. Three years surveillance of viral etiology of acute lower respiratory tract infection in children from 2007 to 2010. Zhonghua Er Ke Za Zhi 2011; 49: 745-749.

14. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375: 1545-1555.

15. Albuquerque MC, Varella RB, Santos N. Acute respiratory viral infections in children in Rio de Janeiro and Teresópolis, Brazil. Rev Inst Med Trop Sao Paulo 2012; 54: 249-255.

16. Lin TY, Huang YC, Ning HC, Tsao KC. Surveillance of respiratory viral infections among pediatric outpatients in northern Taiwan. J Clin Virol 2004; 30: 81-85.

17. Phuong HL, Nga TT, van Doornum GJ, et al. Viral respiratory tract infections among patients with acute undifferentiated fever in Vietnam. Southeast Asian J Trop Med Public Health 2010; 41: 1116-1126.

18. Faghihloo E, Rezaie F, Salimi V, et al. Molecular epidemiology of human respiratory syncytial virus in Iran. Acta Virol 2011; 55: 81-83.

19. Chan PW, Chew FT, Tan TN, Chua KB, Hooi PS. Seasonal variation in respiratory syncytial virus chest infection in the tropics. Pediatr Pulmonol 2002; 34: 47-51.

20. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009; 360: 588-598.

21. Bicer S, Giray T, Çöl D, et al. Virological and clinical characterizations of respiratory infections in hospitalized children. Ital J Pediatr 2013; 39: 22.

22. Broor S, Parveen S, Bharaj P, et al. A prospective three-year cohort study of the epidemiology and virology of acute respiratory infections of children in rural India. PLoS One 2007; 2: e491.

23. Shafik CF, Mohareb EW, Yassin AS, et al. Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age. BMC Infect Dis 2012; 12: 350.

24. Lina B, Valette M, Foray S, et al. Surveillance of community-acquired viral infections due to respiratory viruses in Rhone-Alpes (France) during winter 1994 to 1995. J Clin Microbiol 1996; 34: 3007-3011.

25. Malekshahi SS, Azad TM, Yavarian J, Shahmahmoodi S, Naseri M, Rezaei F. Molecular detection of respiratory viruses in clinical specimens from children with acute respiratory disease in Iran. Pediatr Infect Dis J 2010; 29: 931-933.

26. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. J Med Virol 2009; 81: 750-756.

27. Rosa MR, Ignotti E, Hacon SS, Castro H. Análise das internações por doenças respiratórias em Tangará da Serra-Amazônia Brasileira. J Bras Pneumol 2008; 34: 575-582.

28. Weigl JA, Puppe W, Belke O, Neusüss J, Bagci F, Schmitt HJ. The descriptive epidemiology of severe lower respiratory tract infections in children in Kiel, Germany. Klin Padiatr 2005; 217: 259-267.

29. Kim SH, Huh JH, Bae SY, et al. Epidemiology of respiratory viral infection in 2004-2006. Korean J Lab Med 2006; 26: 351-357.