
EXPERIMENTAL WORKS

Viral Infections in Patients with Acute Respiratory Infection in Northwest of Iran¹

Nasim Asadi Faezi^a, Abed Zahedi Bialvaei^b, Hamed Ebrahimzadeh Leylabadlo^a, Hossein Soleimani^c, Mehdi Yousefi^b, and Hossein Samadi Kafil^{d, *}

^a*Infectious and Tropical Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

^b*Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

^c*Faculty of Medicine, Islamic Azad University, Tabriz, Iran*

^d*Drug Applied Research Center, Faculty of Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*

**e-mail: Kafilhs@tbzmed.ac.ir*

Abstract—Introduction: Acute respiratory infection (ARI) is one of the main causes of morbidity and mortality all around the world. The aim of this study was to determine the frequency, mortality and association with clinical entities of influenza virus type A, influenza virus type B, respiratory syncytial virus (RSV), coronavirus, and adenoviruses in patients with ARI. Materials and Methods: During September 2014 till May 2015, 143 respiratory inpatients samples for viral testing collected from central Hospital in Northwest of Iran. A real-time reverse transcription-PCR (RT-PCR) assay was done to allow in one test the detection of influenza A and B viruses. Also, RSV and adenovirus were identified by Immunochromatography test. Results: Twenty-four (46%) cases were positive for influenza A, which 11 (46%) of them were subtype H1N1 and 13 (54%) cases were subtype H3N2. Also, 21 (40%) cases were positive for influenza B, 5 (10%) cases were positive for RSV, and 2 (4%) cases were positive for adenovirus. One of the patients was positive for both influenza A and adenovirus. Two of the patients were positive for both influenza A and RSV. None of the patients were positive for coronavirus. Conclusions: Our findings show the importance of influenza virus type A, influenza virus type B, RSV, and adenoviruses associated with ARI in hospitalized patient and the different epidemiological patterns of the viruses in Tabriz, Iran.

Keywords: acute respiratory infections, influenza virus, influenza, respiratory syncytial virus, coronavirus, adenoviruses

DOI: 10.3103/S0891416816030046

INTRODUCTION

Acute respiratory infection (ARI) caused by influenza virus type A (subtypes H1N1 and H3N2), influenza virus type B, respiratory syncytial virus (RSV), coronavirus, and adenovirus are major causes of upper and lower respiratory tract diseases in infants and young children, causing croup, bronchiolitis, and pneumonia [1]. ARI is one of the main causes of morbidity and mortality all around the world. However, due to social, nutritional and environmental factors the risk of death due to ARI is 30 times higher in developing countries than in the northern hemisphere [2]. While rarely causing death in industrialized countries, ARIs cause enormous direct and indirect health care costs [3]. In a relatively large proportion of samples obtained from persons suffering from ARI, no pathogens can be detected despite the use of a wide range of sensitive diagnostic assays; even in the most comprehensive studies, a causative agent (either viral or non-viral) can be identified in only 38–85% of the patients

[4, 5]. According to the WHO reports, causes about 4 million annual deaths of children under 2 years and about 20 to 40 percent of hospitalized children at the health centers are associated with acute respiratory infections [6, 7].

Major outbreaks of influenza are always associated with influenza virus type A or B, especially influenza type A virus which has been accounted for four worldwide pandemics during the twentieth century. Due to virus evolution and migration, the transmission of respiratory viruses between animals and humans has been a very severe matter. Because influenza virus infection is a highly contagious respiratory disease that can spread easily by droplets or nasal secretions and is responsible for considerable morbidity and mortality [8]. It is estimated that influenza A viruses cause the death of approximately half a million individuals worldwide every year [9]. It associated with illness among all age groups, but the risk of serious illness and death following infection is highest among persons aged ≥65 years and persons with certain chronic medical conditions. Also, RSV is known for its tendency to

¹ The article is published in the original.

cause bronchiolitis in infancy, but it can infect all age-groups causing illness indistinguishable from influenza [10]. Although RSV infection is the most important viral cause of severe acute lower respiratory tract infection, only a small proportion of children infected with this virus develop severe disease. Increased understanding of the virus and the host response to it, has resulted in expanded therapeutic and preventive options. Adenoviruses also produce a variety of serious diseases in people of all ages. The mode of transmission of adenovirus infections includes respiratory, fomite, droplet, venereal, and faecal-oral routes. They have been shown to spread with ease in AIDS patients, in young children and in hospitalized patients. A constant rate of about 8% of world-wide reported virus infections were observed to be due to adenovirus infections [11]. In the case of coronavirus, many isolates have been reported to cause upper respiratory tract infections in both children and adults [12]. Coronaviruses cause wheezing in young asthmatic children but only rarely cause wheezing in normal children.

Determining etiological diagnoses for patients admitted to the hospital with respiratory symptoms remains a clinical and laboratory challenge. Infections with respiratory viral infections are traditionally diagnosed using viral culture, rapid antigen test, and direct immunofluorescence (DIF) tests [13]. However, over the last 2 decades, nucleic acid amplification techniques, particularly real-time PCR, have become available as diagnostic tools due to its high sensitivity and rapid turnaround time [14]. Aim of the present study was to determine the frequency and mortality of the viral respiratory infections including influenza virus type A, influenza virus type B, respiratory syncytial virus (RSV), coronavirus, and adenoviruses in patients with ARI that referred to central Hospital in Northwest of Iran during September 2014 till May 2015.

MATERIALS AND METHODS

Sample Collection

From September 2014 till May 2015, 143 respiratory specimens for viral testing were collected from central Hospital in Tabriz, Northwest of Iran. The specimens included nasal washes in saline, nasal or nasopharyngeal swabs in viral transport medium, bronchoalveolar lavage throat or oropharyngeal swabs, tracheal aspirates, or sputum. Patient's selection was consecutive and samples were included two respiratory samples collected in special sample collection tubes. All patients had acute onset of respiratory symptoms accompanied by fever and a recent travel history to countries with sustained human-to-human transmission of RSV, coronavirus, adenovirus, influenza virus type A and B, and were analyzed with further assay.

After obtaining written informed consent from the parents, we collected demographic data, information

on the nature and course of illness, medication, complications, duration of stay, parental absenteeism, and follow-up information. Information was supplemented by review of medical records and structured telephone interviews. Data collected from the hospital database included the number of referrals to the central Hospital in Northwest of Iran, the number of acute admissions, and the discharge diagnoses for referrals and admissions.

Influenza Type-Specific Real-Time RT-PCR Assays

Real-time RT-PCR was done based on WHO protocol [6]. Viral RNA was extract from clinical specimens with QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. For each test sample and positive and negative controls, Real-time ready RNA Virus Master Kit (Roche, Germany) was prepared. After Adding 5 μ L viral RNA to the above mix, one-step real-time RT-PCR was performed according to the following conditions: The reverse transcription for 8 minutes at 50°C followed by 30 s at 95°C, 45 cycles of 95°C for 1 s, 56°C for 20 s, 72°C for 1 s, then 1 cycle of 40°C for 30 s using the Rotor-Gene 3000 (Corbett Research, Sydney, Australia) and were analyzed by using a software (Rotor-Gene Version 6.1, Corbett, Australia). The primers and probes were used as described in WHO protocol [6].

RSV, Adenovirus and Coronavirus Identification

RSV and adenovirus were identified by Coris-bio Concept Immunochromatography Diagnosis test (Coris bioConcept, Gembloux, Belgium) for detection in swabs and nasopharyngeal secretions. Every sample was diluted in test buffer and strips were added and final results were compared with positive controls (RSV and respiratory adenovirus positive controls were provided by Coris-bioConcept). Also, sample of suspected patients for coronavirus were sent to center of infection control for identifying by allele specific RT-PCR method (Ministry of Health, Tehran, Iran).

Statistical Analysis

Data were analyzed by the χ^2 test or Fisher's exact test. Spss version 21 was used for statistical analysis.

RESULTS

From 143 patients that referred to central Hospital in Northwest of Iran during the 9-month period, 69 (48%) cases obtained from females and 74 (52%) cases obtained from males. Patients were investigated when clinical signs for viral infection were positive including radiographic signs, fever and etc. Among suspected patients, 91 (64%) samples were negative for all viruses that we have studied and 52 (36%) samples were posi-

The distribution of samples according to sex and the presence of viral agents

	Positive*	H1N1	H3N2	Influenza B	RSV	Adenovirus	Coronavirus
Total	55 (36%)	11 (46%)	13 (54%)	21 (40%)	7 (10%)	3(6%)	0
Male	26 (47.3%)	4 (36%)	7 (54%)	8 (38%)	6 (85%)	1 (34%)	0
Female	29 (52.7%)	7 (64%)	6 (46%)	13 (62%)	1 (15%)	2 (66%)	0
Dead patients	8 (15%)	4 (50%)	0	4 (50%)	0	0	0

* Includes all viruses that are mentioned in the study.

tive at least for one virus. The distribution of samples according to sex and the presence of viral agents have shown in table. Fifty (35%) cases of suspected patient were older than sixty, 91 (64%) cases were between 10 to 60, and 2 (1%) cases were younger than 10 years old. In positive cases, Sixteen (31%) of cases were older than sixty, 34 (66%) cases were between 10 to 60 and 2 (3%) cases were younger than 10 years old. Also, 14 (10%) cases of suspected patients were died, which 6 cases of them belonged to negative result for viruses. While 9 (64%) cases of dead ones were older than sixty years old and rest of them were between 10 to 60 years old. Three (6%) cases of positive results were positive for 2 viruses that the first one were positive for both H1N1 and adenoviruses, the second one were positive for both H3N2 and adenoviruses and the third one were positive for both H3N2 and RSV. Among 3 cases that were positive for both viruses, one of them was died. Among the positive results 2 (1%) cases were younger than 10 years old that both of whom were male and were positive for RSV. Sixty six (46%) cases of suspected patient were lived in Tabriz and 76 (53%) cases were lived in countryside and 1 case was lived in Turkey. From six suspicious patients to coronavirus, no one was positive. Also, there was no significant relationship between the presence of viral agents and positive bacterial culture ($p > 0.05$).

DISCUSSION

The study showed that among the all cases of respiratory infection, 46% cases were positive for influenza A that 46% of them were subtype H1N1 and 54% cases were subtype H3N2. Also, 40% of cases were positive for influenza B. In the case of RSV and adenovirus, 13% and 6% of cases were positive, respectively. In spite of other viruses, no positive results were observed for coronavirus. These reveal the importance of above mentioned viruses, which associated with ARI in hospitalized patient and the different epidemiological patterns in Tabriz, Iran. A study that carried out in Kerman province revealed that from 168 specimens, 18 cases (10.7%) were infected with adenovirus and 63 cases (37.5%) with RSV [7]. This rate is slightly higher than the result obtained in this study that may be because their study was conducted on children under 2 years. In another study that conducted in a Tehran hospital on children with acute respiratory

infection, influenza A virus, RSV, and adenovirus were detected in 4.4, 5.7, and 6.3%, respectively [15]. In the detection of seasonal influenza A viruses using RT-PCR assay during 2009 flu pandemic in Golestan province showed, 13 (8.49%) were infected with seasonal influenza H1N1 and 25(16.33%) with seasonal H3N2 influenza [16]. In their study, the rate of infection with seasonal H1N1 and H3N2 was similar to other studies reported from Iran, but lower than the rate reported from other parts of the world. A survey that conducted during Influenza A (H1N1) pandemic in 2009 showed, of 3672 confirmed cases, 140 (3.8%) deaths were occurred [17]. However, the mortality rate in our study, of 52 suspected patients among all viruses was 8 (15.2%).

Acute respiratory tract infections are responsible for considerable morbidity and mortality in humans and animals, and the costs attributable to acute respiratory tract illnesses in humans are an important burden on national health care budgets [4]. The morbidity and mortality from influenza and RSV vary from year to year, from community to community, and from institution to institution, and an accurate estimate of disease burden requires temporal, geographic, and institutional breadth [18]. According to WHO latest data on H1N1 influenza, at least 13554 deaths related to 2009 H1N1 pandemic have been reported and nearly 883 of them were reported from WHO Regional Office for the Eastern Mediterranean (EMRO) [19]. In studies of respiratory virus detection among children hospitalized with respiratory illness from different parts of the world, RSV and influenza are frequently associated with a substantial proportion of hospitalizations [20]. Although human adenovirus infections are less common in adults than other respiratory virus infections, such as influenza viruses, human rhinoviruses, human parainfluenza viruses, enteroviruses, and human coronaviruses, they can cause fatal respiratory tract infections in affected patients [21].

Influenza occurs all over the world, with an annual global attack rate estimated at 5–10% in adults and 20–30% in children. In temperate regions, influenza is a seasonal disease occurring typically in winter months: it affects the northern hemisphere from November to April with a peak in February and the southern hemisphere from April to September. A cor-

relation between influenza activity and climate has previously been suggested for other countries [22].

Vaccination remains important as a means of reducing the morbidity and mortality caused by influenza viruses. The H1N1 influenza virus, which caused the 2009 pandemic, continues to circulate in some parts of the world, causing variable levels of disease and outbreaks. In some countries, seasonal trivalent vaccines are available that cover the H1N1 (2009) virus. In other countries, however, seasonal influenza vaccines are not available. WHO advises that there is still public health value in using monovalent H1N1 vaccine (where available) to immunize persons at risk of severe disease from H1N1 influenza infection, especially where trivalent seasonal influenza vaccine is not available. However, for individuals who cannot be or have not been vaccinated or when vaccine is not available, antiviral agents can provide an important alternative. Groups at increased risk of severe illness from the pandemic H1N1 virus included young children, pregnant women, and people with underlying respiratory or other chronic conditions, including asthma and diabetes. Patients who have severe or deteriorating influenza should be treated as soon as possible with oseltamivir. Patients who are at higher risk of severe or complicated influenza should be treated with oseltamivir or zanamivir as soon as possible.

Travelers, like local residents, are at risk during the influenza season. In addition, groups of travelers (e.g. on cruise ships) that include individuals from areas affected by seasonal influenza may experience out-of-season outbreaks. Travelers visiting countries in the opposite hemisphere during the influenza season are at special risk, particularly if they do not have some degree of immunity through recent infection or regular vaccinations. In this study, almost half of the patients were living in small towns or rural areas with different climatic conditions and different prevalence of viral diseases. These patients are often referred for treatment to central Hospital in Northwest of Iran and may have a role in the transmission of the disease to new areas. Given the non-specific symptoms of these viruses and the high prevalence of viral diarrhea in our region, more laboratories should be equipped for virus detection and vaccination might be considered as a prevention strategy. To detection of influenza viruses, PCR method widely used, which its materials and equipment's is available in many countries. However, the rate of mutation in the HA and NA genes of the influenza virus is high. Therefore to determine the subtypes, we used to specific primers that suggested by CDC. Also, real-time PCR considerably increases the diagnostic yields for respiratory viruses from patients admitted with respiratory symptoms within a clinically relevant time frame. This allows clinicians to initiate optimal patient management and to initiate adequate (future) use of antiviral therapy and optimal infection control [23]. Therefore, we can conclude that: (a) in spite of general conception, the viruses that investi-

gated in our study have shown endemic distribution among the study period. Because of spreading rumors about Ebola disease outbreaks on social networking, a wave of fear in people who had similar and even mild symptoms were created. However, lastly it considered as an epidemic of influenza or possible coronavirus. (b) Our results indicated that rapid test method which was used in the present study showed less positive results in compare with RT-PCR method, (c) in the case of influenza B, we had the same mortality or more than influenza A which possibly is in contrary with others, (d) finally, we should conclude that we need to develop local protocols and strategies in the case of viral infections, and how we have to act when we face with micro-epidemics' and endemics to prevent more distribute of the infection.

ACKNOWLEDGMENTS

The authors would like to thank all staff of Microbiology labs collaborated in this study. Also Authors thank Mr Nikmaram in central Hospital in Tabriz-Northwest of Iran for help on sample collection. This study was granted by Drug applied research Center, Tabriz University of Medical Sciences.

REFERENCES

1. Hall, C.B., Respiratory syncytial virus and parainfluenza virus, *N. Engl. J. Med.*, 2001, vol. 344, pp. 1917–1928.
2. Videla, C., Carballal, G., Misirlian, A., and Aguilar, M., Acute lower respiratory infections due to respiratory syncytial virus and adenovirus among hospitalized children from Argentina, *Clin. Diagn. Virol.*, 1998, vol. 10, pp. 17–23.
3. Gröndahl, B., Puppe, W., Hoppe, A., Kühne, I., Weigl, J.A., and Schmitt, H.-J., Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study, *J. Clin. Microbiol.*, 1999, vol. 37, pp. 1–7.
4. Fouchier, R.A., Hartwig, N.G., Bestebroer, T.M., et al., A previously undescribed coronavirus associated with respiratory disease in humans, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, vol. 101, pp. 6212–6216.
5. Jain, S., Self, W.H., Wunderink, R.G., et al., Community-acquired pneumonia requiring hospitalization among U.S. adults, *N. Engl. J. Med.*, 2015, vol. 373, pp. 415–427.
6. *WHO Information for Molecular Diagnosis of Influenza Virus in Humans—Update*, World Health Organization, 2011.
7. Parizi, M.H.D., Molaie, H.R., Afshar, A.A., and Salari, A.A., The frequency distribution of parainfluenza, adeno and respiratory syncytial virus infections in children below 2 years old with bronchiolitis, by multiplex polymerase chain reaction method, Afzalipoor hospital, Kerman, 2006, *J. Kerman Univ. Med. Sci.*, 2008, vol. 15, pp. 305–311.

8. Templeton, K.E., Scheltinga, S.A., van den Eeden, W.C., Graffelman, W.A., van den Broek, P.J., and Claas, E.C., Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction, *Clin. Infect. Dis.*, 2005, vol. 41, pp. 345–351.
9. Englund, J.A., Antiviral therapy of influenza, *Semin. Pediatr. Infect. Dis.*, 2002, vol. 13, pp. 120–128.
10. Zambon, M., Stockton, J., Clewley, J., and Fleming, D., Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: An observational study, *Lancet*, 2001, vol. 358, pp. 1410–1416.
11. Rubin, B.A., Clinical picture and epidemiology of adenovirus infections (a review), *Acta Microbiol. Hung.*, 1993, vol. 40, pp. 303–323.
12. Isaacs, D., Flowers, D., Clarke, J., Valman, H., and MacNaughton, M., Epidemiology of coronavirus respiratory infections, *Arch. Dis. Child.*, 1983, vol. 58, pp. 500–503.
13. Uyeki, T.M., Influenza diagnosis and treatment in children: A review of studies on clinically useful tests and antiviral treatment for influenza, *Pediatr. Infect. Dis. J.*, 2003, vol. 22, pp. 164–177.
14. van de Pol, A.C., van Loon, A.M., Wolfs, T.F., et al., Increased detection of respiratory syncytial virus, influenza viruses, parainfluenza viruses, and adenoviruses with real-time PCR in samples from patients with respiratory symptoms, *J. Clin. Microbiol.*, 2007, vol. 45, pp. 2260–2262.
15. Barati, M., Talebi-Taher, M., Noorbakhsh, S., Ebrahimi, Taj F., and Tabatabaei, A., Diagnosis of the adenovirus, RSV, and influenza virus by rapid detection test (immunochromatography) in children with acute respiratory infection, *Iran. J. Pediatr. Soc.*, 2010, vol. 2, pp. 79–83.
16. Zhand, S., Tabaraei, A., Fotoohi, F., et al., Detection of seasonal influenza H1N1 and H3N2 viruses using RT-PCR assay during 2009 flu pandemic in Golestan Province, *Med. Lab. J.*, 2014, vol. 8, pp. 27–32.
17. Gouya, M., Nabavi, M., Soroush, M., et al., Mortality from pandemic influenza A (H1N1) in Iran, *Iran. Red Crescent Med. J.*, 2011, vol. 13, p. 698.
18. Ellis, S.E., Coffey, C.S., Mitchel, E.F., Dittus, R.S., and Griffin, M.R., Influenza and respiratory syncytial virus-associated morbidity and mortality in the nursing home population, *J. Am. Geriatr. Soc.*, 2003, vol. 51, pp. 761–767.
19. WHO latest update on H1N1 influenza 2009, Update 83. http://www.who.int/csr/don/2010_01_15/en/index.html.
20. Broor, S., Dawood, F.S., Pandey, B.G., et al., Rates of respiratory virus-associated hospitalization in children aged <5 years in rural northern India, *J. Infect.*, 2013, vol. 13, pp. 1–22.
21. Guo, L., Gonzalez, R., Zhou, H., et al., Detection of three human adenovirus species in adults with acute respiratory infection in China, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2012, vol. 31, pp. 1051–1058.
22. Kyriazopoulou, V., Frantzidou, F., Diza, E., and Souliou, E., Influenza surveillance in N. Greece during 1983–1993, *J. Infect.*, 1995, vol. 30, pp. 167–171.
23. Safaeyan, F., Nahaei, M.R., Seifi, S.J., Kafil, H.S., and Sadeghi, J., Quantitative detection of *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* in patients with new influenza A (H1N1)/2009 and influenza A/2010 virus infection, *GMS Hyg. Infect. Control*, 2015, vol. 10, p. Doc06.