

Influenza A virus among the hospitalized young children with acute respiratory infection. Is influenza A co infected with respiratory syncytial virus?

Seyed Mohammad Alavi (MD)^{*1}
Manoochehr Makvandi (PhD)²
Saied Najafi-Fard (MSc)³
Leila Alavi (VMD)⁴

1- Jundishapur Infectious and Tropical Diseases Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran.
2- Department of Virology, College of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran.
3- College of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran.
4- Food and Drug Deputy of Jundishapur University of Medical Sciences, Ahvaz, Iran.

*** Correspondence:**

Seyed Mohammad Alavi, Infectious and Tropical Diseases Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran.

E-mail:

alavi.seyedmohammad@yahoo.com
Tel: 0098 611 3387724
Fax: 0098 611 3387724

Received: 2 June 2012
Revised: 15 Aug 2012
Accepted: 22 Aug 2012

Abstract

Background: Both influenza A virus (IAV) and respiratory syncytial virus (RSV) cause acute respiratory infection (ARI) in infants and young children. This study was conducted to determine Influenza A virus and its co infection with RSV among the hospitalized children with ARI.

Methods: A total of 153 throat samples of the hospitalized young children aged between below one year and 5 years with the clinical signs of ARI were collected from the different hospitals in Khuzestan from June 2009 to April 2010. The samples were tested for Influenza A viruses by real time PCR. Positive IAV samples were tested for influenza A sub type H1N1 and for RSV by the nested PCR.

Results: In this study, from the total 153 samples, 35 samples (22.9%) including 15 (42.8%) females and 20 (57.2%) males were positive for influenza A viruses. From the 35 positive samples for IAV, 14 were positive for swine H1N1 subtype. All the positive samples for influenza showed negative for RSV infection which revealed no coinfection with RSV. The prevalence of influenza A among age/sex groups was not significant.

Conclusion: Influenza A is a prevalent viral agent isolated from young children with ARI. Influenza A subtype H1N1 was accounted for the 40 percent all laboratory-proven diagnoses of influenza in 2009. No evidence of coinfection of influenza A and RSV has been observed in the present study.

Keywords: Respiratory syncytial virus, Influenza A virus, swine H1N1, Acute respiratory infection, Co-infection.

Caspian J Intern Med 2012; 3(4):530-534

Influenza viruses are members of the orthomyxoviridae family and include influenza virus types A, B, and C. The negative sense RNA genome of influenza A and B virus is composed of eight separate segments, while influenza C virus genome consists of only seven single stranded negative- sense (1). Influenza A virus is zoonotic pathogen capable of infecting birds, swine, horse, felines and other species (1). In human influenza A, subtypes H1N1 and H3N2 viruses are mostly responsible for seasonal epidemics and global pandemic (2).

Respiratory syncytial virus (RSV) is a member of the paramyxoviridae family (3). It infects the epithelium airway and is responsible for the acute respiratory infection (ARI) in infants, young children, asthmatics, and adults (4– 6). RSV is the most common etiology in bronchiolitis (7).

The acute infection of RSV-induced bronchiolitis may lead to the hospitalization of 1–2% of infants and children (8). RSV can be divided into two major antigenic groups, A and B, by nucleotide sequence differences (9, 10). This diversity is mainly found in the G surface glycoprotein (11). There are very few reports on the coinfection of RSV and influenza A virus among the patients (12, 13).

Knowing the influence of RSV and influenza on ARI helps the involved physicians to decide the best antimicrobial agent for managing the patients and preventing the unnecessary antibiotics for respiratory infections. Because of the scanty data about these two viruses in Khuzestan province, southwest of Iran, this study was conducted to determine the influenza A infection and its coinfection with RSV among the hospitalized young children with ARI.

Methods

In this cross sectional study the throat samples of the hospitalized young children with ARI were tested. A total of 153 throat samples of children aged between below one year and 5 years with the clinical signs of ARI were collected from June 2009 to April 2010 in the different hospitals across the Khuzestan province, southwest of Iran. The diagnosis of ARI was based upon the National ARI guideline and by the pediatric or trained general physicians. The samples were tested for influenza A viruses by real time polymerase chain reaction (PCR), and if the samples were positive for influenza A were tested for swine H1N1 subtype and for RSV by nested PCR. The specimens in viral transport media were transferred to our laboratory in the Virology Department of Ahvaz Jundishapur University of Medical Sciences. All the samples were kept at -70°C prior to test.

Real time PCR for Influenza A: The RNA extraction was carried out for all the throat samples using the high pure viral nucleic acid kit (Roche, Germany), cDNA was then synthesized from the extracted RNA, followed by influenza A detection using the real time PCR test. The following primers were used for the detection of influenza A virus (14):

Inf A Forward GAC CRA TCC TGT CAC CTC TGA C

Inf A Reverse AGG GCA TTY TGG ACA AAK CGT CTA

Inf A Probe TGC AGT CCT CGC TCA CTG GGC ACG

TaqMan probes are labeled at the 5'- end with the reporter molecule 6- caboxyfluorescein (FAM) and with the quencher, Blackhole Quencher1 (BHQ1) at the 3'- end. The 25 µl of the master mix including 12 µl one step master mix (ABI ,USA) , 5 µl of the extracted RNA ,0.5 µl of 40 µmol of the each forward , reverse primers and probe , 0.5 µl RT and 6µl of the D/w was prepared and subjected to ABI (Applied Biotechnology) one step real time PCR. The

following condition was programmed for Real time PCR. 42 °C for 60min (for cDNA preparation) 95 °C for 15 minutes followed by 45 cycles of 95 °C for 20 sec and 60 °C for 40 sec. Real-time RT-PCR was carried out by the human influenza A H1N1 specific primers and probes on those samples that were positive for influenza A virus.

Nested PCR for RSV: The prepared cDNA of each sample was tested by nested PCR to detect RSV and the following conserved G region primers were used (15).

G1- CCA TTC TGG CAA TGA TAA TCT C

G2- GTT TTT TGT TTG GTA TTC TTT TGC GA

G3- CGG CAA ACC ACA AAG TCA CAC

G4- GGG TAC AAA GTT AAA CAC TTC

The primers G1 and G2 were used for the first round. The 25 µl of PCR master mix containing 5 µl of the cDNA of the each sample, 12.5 of the 1X PCR master mix, 50 pmol the each G1 and G2 primer was added to master mix. The PCR was performed for 40 cycles in Techne Thermal cycler UK, consisting initially 5 min for 95 °C followed by 1 min at 52 °C ,2 min at 72C° ,1 min at 95 °C and finally 5 min at 72 °C for one cycle. The primers G3 and G4 were used for the second round. The 25 µl of PCR master mix containing 5 µl of the cDNA of the each sample, 12.5 of the 1X PCR master mix, 50 pmol of the each G3 and G4 primer was added to master mix. The PCR was performed for 35 cycles consisting initially 5 min for 95 °C followed by, 1 min at 60 °C , 1 min at 72 °C ,1 min at 95 °C and finally 5 min at 72 °C for one cycle. The expected final PCR product was 326 BP (15).

Statistical analysis: Statistical analysis was performed on PCR results, data were analyzed in SPSS version 15. Differences with p value <0.05 were considered significant.

Results

From the total 153 samples, 35 samples (22.9%) including 15 (42.8%) females and 20 (57.2%) males were positive for influenza A viruses. The mean age of the male patients was 2.57±2.21 years and the mean age of the female was 3.01±1.87 years. The patients were from below the age of one to 5 years. From the 35 positive samples for IAV, 14 were positive for swine H1N1 sub type. All the positive samples for IAV showed negative for RSV infection which revealed no coinfection with RSV. The distribution of influenza A among the different age groups is shown in table 1.

Table 1: Distribution of influenza A positive patients among the different age groups.

Age (month)	0-11(N=34)		12-23(n=51)		24-59(n=68)		total(n=153)	
	Flu A+	Flu A-	Flu A+	Flu A-	Flu A+	Flu A-	Flu A+	Flu A-
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Male	4 (25)	12 (75)	6 (23)	20 (77)	10 (21.7)	36 (78.3)	20 (22.7)	68 (77.3)
Female	1 (5.6)	17 (94.4)	7 (28)	18 (72)	7 (31.8)	15 (68.2)	15 (23.7)	50 (76.93)
Total	5 (14.7)	29 (85.3)	13 (25.5)	38 (74.5)	17 (25)	51 (75)	35 (22.8)	118 (77.2)

The prevalence of influenza A among age/sex groups (table 2) was not found significantly different ($p>0.05$). As shown in table 3, a total number of confirmed H1N1 cases above 2 years old children was greater than those below 2 years ($p<0.05$). The distribution of age groups in the studied children, confirmed cases of influenza A and H1N1 cases are shown in figure 1.

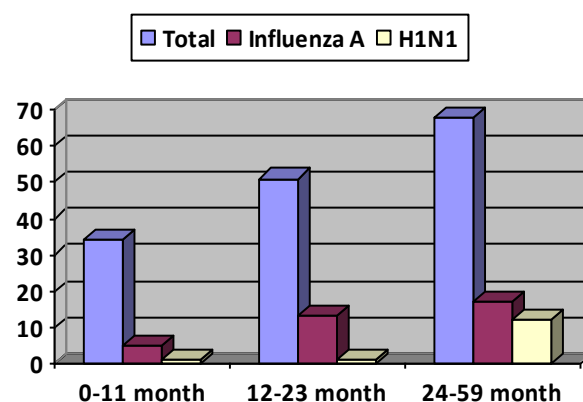
Table 2: comparison of age and sex group of young children with or without influenza A

	Flue A +	Flue A -	P value
	N=35	N=118	
	N (%)	N (%)	
Sex			0.55
Male	20 (57.1)	68 (57.6)	
Female	15 (42.9)	50 (42.4)	
Age (month)			0.29
0-11	5 (14.3)	29 (24.6)	
12-23	13 (37.1)	38 (32.2)	
24-59	17 (48.6)	51 (43.2)	

Table 3: comparison of age and sex group of influenza A infected young children with or without H1N1

	H1N1 +	H1N1 -	P value
	N=14	N=21	
	N (%)	N (%)	
Sex			0.36
Male	9 (64.3)	11 (52.4)	
female	5 (35.7)	10 (47.6)	
Age (month)			0.0004
0-11	1 (7.1)	4 (19.1)	
12-23	1 (7.1)	12 (57.1)	
24-59*	12 (85.8)	5 (23.8)	

*Statistically significant

**Figure 1: Distribution of age groups in studied children 1-5 years old.**

Discussion

Previous studies have indicated that hospitalization of children younger than five years of age due to influenza is considerable; the rate of this hospitalization is similar to the rates of influenza hospitalization among older adults. However, the rates of hospitalization attributable to laboratory-confirmed influenza infections are not well described (16). The present study has shown that about 30% of ARI cases in young children are associated with influenza A infection. This means that Influenza A may be the major cause of ARI among young children in the region of this study. Our results are in agreement with other studies indicating influenza A as the most important viral pathogen inducing ARI (2, 12, 17). In poehling et al.'s study the researchers reported the rate of 28% for the inpatient children who had laboratory-confirmed influenza (18). Although published studies in Iran about the influence of influenza on respiratory illness among children are very few but according to few reports, influenza is a serious threat for Iranian young children (19).

We found no relation between sex and influenza A infection. This finding is inconsistency with previous studies

(20). There are also reports on the effect of sex on influenza infection. Poehling et al. reported that females are at the higher risk of acute IAV infection than the males (18). In our study, IAV infection in different age groups was not significantly different. This finding is in agreement with Neuzil et al. who reported similar rate of IAV infection between those less than 2 years old children and older patients (16).

The present study revealed that 40% of influenza A among the young children at the time of the study were attributed to subtype swine H1N1. In contrast to seasonal influenza, H1N1 is more frequent in children above / less than 2 years old. In the present study, no coinfection of influenza A virus and RSV was found. The frequency of coinfection of influenza A virus with RSV and or coinfection with the different subtypes of influenza virus or different subtypes of RSV is not well-documented in the literature and is essentially unknown, although it is likely to be low (21). The reason for this phenomenon is not clear to us but we believe that this status may be due to the protective effect of influenza virus against RSV infection previously discussed by Walzl et al. (12). There are limited reports about RSV/Influenza coinfection with controversy results (12, 13). The role of the other viral agents for acute respiratory disease among the infants and children including adenoviruses, parainfluenza, rhinovirus, corona viruses, metapneumovirus have not been studied in the present study, however it requires further investigation.

In our study, some limitations and weaknesses should be considered. Our study is hospital-based, it may be biased. This bias may result in the underestimation of influenza in children. The other source of bias included the influenza subgroups when we restricted our study to H1N1, however, the other subgroups might have been mistaken in our study. Future population based-studies are recommended.

In conclusion influenza A is a prevalent viral agent isolated from the young children with ARI. Influenza A subtype H1N1 was accounted for 40 percent of all laboratory-proven diagnoses of influenza in 2009. No evidence of coinfection of influenza A and RSV has been observed in the present study.

Acknowledgments

We wish to thank Dr. Mokhtari, Faculty of Health, University of Tehran Iran, for providing RSV (prototype

RSV group A and B strains) and Influenza A strain. Dr Gooya, Dr Soroosh, CDC, the Ministry of Health of Iran for the kind cooperation, the personnel and the Chief of the Infectious and Tropical Diseases Research Center of Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran for the approval this study.

Funding: This study is funded by Jundishapur Infectious and Tropical Diseases Research Center.

Conflict of interest: None declared

References

1. Shaw ML, Palese P. Orthomyxoviridae: The viruses and their replication. In: Hoowley DM. Field Virology. 5th ed. Philadelphia, USA: Lippincott William & Wilkins 2007; pp: 1647-89.
2. Barnard DL. Animal models for the study of influenza pathogenesis and therapy. *Antiviral Research* 2009; 82: A110-22.
3. Sullender WM. Respiratory syncytial virus genetic and antigenic diversity. *Clin Microbial Rev* 2000; 13: 1-15.
4. Leader S, Kohlhasse K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. *J Pediatr* 2003; 143: S127-32.
5. Holt PG, Sly PD. Interactions between RSV infection, asthma, and atopy: unraveling the complexities. *J Exp Med* 2002; 196: 1271-5.
6. Falsey AR, Formica MA, Walsh EE. .Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription-PCR to viral culture and serology in adults with respiratory illness. *J Clin Microbiol* 2002; 40: 817-20.
7. Psarras S, Papadopoulos NG, Johnston SL. Pathogenesis of respiratory syncytial virus bronchiolitis-related wheezing. *Paediatr Respir Rev* 2004; 5: S179-84.
8. Boyce T, Mellen B, Mitchel EF Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in medicaid. *J Pediatr* 2000; 137: 865-70.
9. Sullender WM, Mufson MA, Anderson LJ, Wertz GW. Genetic diversity of the attachment protein of subgroup B respiratory syncytial viruses. *J Virol* 1991; 65:5425-34.
10. Storch GA, Anderson LJ, Park CS, Tsou C, Dohner DE. Antigenic and genomic diversity within group A respiratory syncytial virus. *J Infect Dis* 1991; 163: 858-61.

11. Cane PA, Matthews DA, Pringle CR. Identification of variable domains of the attachment (G) protein of subgroup A respiratory syncytial viruses. *J Gen Virol* 1991; 72: 2091–6.
12. Walzl G, Tafuro S, Moss P, Openshaw PJ, Hussell T. Influenza virus lung infection protects from respiratory syncytial virus-induced immunopathology *J Exp Med* 2000; 192: 1317-26.
13. Shinjoh M, Omoe K, Saito N, Matsuo N, Nerome K. In vitro growth profiles of respiratory syncytial virus in the presence of influenza virus. *Acta Viral* 2000; 44: 91-7.
14. World Health prganization. CDC protocol of real-time RTPCR for swine influenza A (H1N1). 2009; Availbale at:[http://www.who.int/csr/resources/publications/swineflu/CDC_realtime RTPCR_protocol-20090428.pdf](http://www.who.int/csr/resources/publications/swineflu/CDC_realtime_RTPCR_protocol-20090428.pdf). Accessed July 24, 2012.
15. Johnson PR, Spriggs MK, Olmsted RA, Collins PL. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: Extensive sequence divergence between antigenically related proteins. *Proc Natl Acad Sci U S A* 1987; 84: 5625–9.
16. 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: 225-31.
17. Nicholson KG, McNally T, Silverman M, Simon P, Zambon MC. Influenza related hospitalization among children in Leicestershire. *Pediatr Infect Dis J* 2003; 22: S228-230.
18. Poehling KA, Edwards KM, Weinberg GA, et al. The Under recognized Burden of Influenza in Young Children. *N Engl J Med* 2006; 355: 31-40.
19. Soleimani GR, Akbar poor M. Clinical presentation of novel influenza (H1N1) in hospitalized children. *Iran J Pediatr* 2011; 21: 200-4.
20. Thorner AR. Clinical manifestations, and diagnosis of pandemic H1N1 influenza ('swine Influenza'). Available at:[http://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of – pandemic- h1n1-influenza-swine- influenza](http://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-pandemic-h1n1-influenza-swine-influenza). Accessed Dec 27, 2009.
21. Falsey AR, Cunningham CK, Barker WH, et al. Respiratory syncytial virus and Influenza A infections in the hospitalized elderly. *J Infect Dis* 1995; 172: 389–94.