

Original Article

Active Surveillance of Influenza A and Other Respiratory Viruses in Children with Influenza-like-illness in Two Seasons

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Abstract

Introduction: The aim of this study was to assess the surveillance of influenza A/other respiratory viruses and risk factors in hospitalized children with the symptoms of influenza-like illness during two consecutive influenza seasons.

Methodology: All children hospitalized with a diagnosis of influenza-like illness had been investigated for Influenza A and other respiratory antigens in pharyngeal/nasopharyngeal secretions.

Results: A total of 132 hospitalized children between December 2013-May 2014 and December 2014-May 2015 were enrolled in this study. At least one respiratory virus was found to be positive by RT-PCR in 78 (59%) patients, influenza A (H3N2) was detected in only 8 (6%) patients. In 54 (41%) patients samples no respiratory viral pathogen was detected and in 70 (53%) patients, one non-influenza A virus was detected. The respiratory viral pathogens detected in decreasing rates were: RSV (n = 46, 35%), HCoV (n = 10, 7.5%), adenovirus (n = 7, 5%), rhinovirus (n = 6, 4.5%), HMPV (n = 5, 4%), Influenza B (n = 4, 3%) ve human Bocavirus (n = 2, 1.5%). In 10 patients, coinfection was detected, however none was with H3N2. In the H3N2 (+) group, the following risk factors were identified: age older than three years (p < 0.05), asthma history (p < 0.05) and chronic lung diseases (p < 0.05).

Conclusion: Influenza A virus was detected in 6% of hospitalized patients with influenza-like illness. Viruses other than Influenza, especially RSV, can cause similar symptoms compatible with influenza-like-illness.

Key words: Influenza A; influenza like illness; respiratory viruses.

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Introduction

Acute respiratory tract infections (ARTIs) are common in children; mostly they are self-limiting diseases however they can cause respiratory failure which can result in critical care admission. They are still the leading causes of morbidity and mortality in children [1]. Many pathogenic microorganisms may cause ARTIs, however viruses are the most commonly identified agents [2]. Respiratory syncytial virus (RSV), Influenza A and B, human rhinovirus (HRV), adenovirus, human metapneumovirus (HMPV), human coronavirus (HCoV-229E and HCoV OC43) are the most frequent of the known causative viruses. Recently described viruses such as HCoV-NL63 and human Bocavirus (HBoV) are also responsible for ARTIs.

Among these viruses, Influenza, which is a vaccine preventable disease, is one of the most commonly associated with severe infection. It causes outbreaks every year, especially in the winter season.

Influenza virus belongs to the orthomyxoviruses family and has three types named A, B and C. Influenza virus type A and B cause epidemic disease and the associated antigens are included in influenza vaccines. Type C influenza virus is associated with mild influenza-like illness and causes sporadic disease. It is estimated that, every year 15-42% of preschool and school-age children are infected with influenza virus [3]. The severe disease is associated with low-income settings in children and 99% of deaths in children occur in developing countries [4,5]. The other well-known

risk factors for severe infection are: under two years old; underlying chronic diseases; immunosuppression; long term aspirin use; and morbid obesity [6]. It is estimated that each year Influenza causes 500,000 deaths worldwide [7].

Influenza-like-illness is a clinical diagnosis which can be used as a replacement for influenza disease in epidemiological researches. In surveillance programmes for influenza The Centers for Disease Control and Prevention (CDC) suggest using the criteria fever plus cough or sore throat for ILI diagnosis [8]. The European Centre for Disease Prevention and Control (ECDC) define ILI as; sudden onset of symptoms and at least one of the following: i) fever or feverishness, ii) malaise, iii) headache, iv) myalgia and at least one of the following three respiratory symptoms: i) cough, ii) sore throat, iii) shortness of breath [9]. All these symptoms are also associated with other viral ARTIs.

The main aim of this study was to determine the surveillance and the risk factors of Influenza A, during two influenza seasons, in hospitalized children with symptoms of influenza-like-illness. We also investigated the other respiratory viruses simultaneously.

Methodology

The Global Influenza Hospital Surveillance Network was established in 2012 to obtain valid epidemiologic data on hospital admissions with influenza-like illness in consecutive seasons in different

countries [10-13]. One of the participating hospitals of this multi-center, prospective, active surveillance study is our hospital, which is a tertiary care training and research hospital in Istanbul, Turkey. In this study, we used the data collected from pediatric patients of our hospital included in this study in 2013-2014 and 2014-2015 influenza seasons lasting from December to May.

The aims of this study were;

- i) Hospital based surveillance of Influenza A as done in many countries,
- ii) Effect of Influenza vaccination,
- iii) To determine other different respiratory viruses except Influenza A in hospitalized children with ILI.
- iv) To assess the possible risk factors for ILI.

A total of 189 patients with the diagnosis of ICD code in Table 1 or Table 2 were hospitalized. Firstly, the patients were investigated if they can be accepted as resident population. If so, inclusion criteria were investigated.

Inclusion criteria were, patients hospitalized during last 24-48 hours with the diagnosis of ICD codes as shown in Table 1 and 2 were determined by hospital records. These children were investigated for ILI and the patients with eligible criteria were included in the study. Inclusion criteria for patients aged 5 years and older with influenza-like illness were; at least one of the following: i) temperature $\geq 37.8^{\circ}\text{C}$, ii) headache, iii) myalgia, iv) malaise and at least one of the following: i) cough, ii) sore throat, iii) shortness of breath during the last seven days of admission according to ECDC

Table 1. International Classification of Diseases Code version 10 (ICD 10).

Patients >5 years	International Classification of Diseases Code version 10 (ICD 10)
Acute respiratory infection	J00-J06, J20-J22, H66.90
Acute myocardial infarction or acute coronary syndrome	I20-I25.9
Asthma	J45.2-J45.22, J45.9-J45.998, J44-J44.9
Heart failure	I50-I50.9; I51.4
Pneumonia and influenza	J09-J18
Chronic Pulmonary Obstructive disease	J40-J44.9
Myalgia	M79.1
Metabolic failure (diabetic coma, renal dysfunction, acid-base disturbances, alterations to the water balance)	E11.9, E10.9, E11.65, E10.65, E10.11, E11.01, E10.641, E11.641, E10.69, E11.00, E10.10, E11.69, N17.0, N17.1, N17.2, N17.8, N17.9, N18.1, N18.2, N18.3, N18.4, N18.5, N18.6M N18.9, N19, E87.0, E87.1, E87.2, E87.3, E87.4, E87.5, E87.6, E87.70, E87.71, E87.79, E86.0, E86.1
Altered consciousness, convulsions, febrile-convulsions	R40.20, R40.4, R40.0, R40.1, R56.00, R56.01
Dyspnea/respiratory abnormality	R06.0, R06-R06.9
Respiratory abnormality	R06.9
Shortness of breath	R06.02
Respiratory abnormality	R06.3, R06.00, R06.09, R06.83
Respiratory symptoms/chest symptoms	R06.89

definition [9]. Children under five years who had one of these symptoms during the last seven days of admission were also included. Resident population was defined as, patients who were living in Asian part of İstanbul during the last six months.

Exclusion criterias were, i) patients hospitalized during the last one month, ii) Patients of the parents / legal guardians whom did not agree to give consent, iii) children who live in child hostels, iv) patients whom are not accepted as resident population.

Parents / legal guardians were informed of the study and informed consent was obtained before inclusion of all cases. Physician assistants who filled the questionnaire were educated.

All the children hospitalized with the diagnosis of ILI into our Pediatrics departments had been investigated for Influenza A and other respiratory antigens in pharyngeal / nasopharyngeal secretions. Pharyngeal / nasopharyngeal samples were obtained with the standard method of collection[14].

Date of birth weight, gestational age, duration of breastfeeding, hospitalization in the past year, hospital admission in the last three months, immunization with influenza vaccine, underlying chronic diseases; immunocompromised status, malignancy, renal insufficiency, asthma, chronic lung diseases and neuromuscular diseases were questioned from the patients with the diagnosis of ILI. The study protocol was approved by the Ethics Committee of İstanbul University İstanbul Medical Faculty.

Microbiology

Nasal or nasopharyngeal swabs were collected using Virocult (Medical Wire & Equipment, Corsham, Wiltshire, UK) and sent to the laboratory in 1-3 days. Upon arrival in the laboratory, all samples were

transferred to cryo-tubes and if not tested on day of arrival they were stored at 4-8°C.

EZ1 Virus mini kit V2.0 (Catalog number: 955134, Qiagen, HildenGermany) was used for total nucleic acid extraction. Real-time PCR based, multiplex FTD Respiratory Pathogens 21 kit (Fast-track diagnostics Ltd. Valletta-Malta) was used for detection of respiratory pathogens on RotorGene Q platform (Qiagen, HildenGermany). Kit is able to detect following pathogens: influenza A, H1N1, influenza B, rhinovirus, coronavirus NL63, 229E, OC43, HKU1, parainfluenza 1, 2, 3, 4, human metapneumovirus A/B, bocavirus, Mycoplasma pneumoniae, respiratory syncytial virus A/B, adenovirus, enterovirus, parechovirus and internal control.

For detection of Influenza H3 subtype, Influenza B Yamagata and Victoria lineages real-time RT-PCR method was performed using an ABI 7500 platform with CDC primers and probes according to the CDC protocol [15].

Statistical analysis

Statistical calculations were performed with NCSS 11 data Analysis (Number Cruncher Statistical System), 2007 for Statistical Software (Utah, Corporation, Kaysville, USA). Besides descriptive statistical calculations (mean and Standard deviation and frequency), Tukey test, Pearson chi-square test, Fischer’s exact tests were used for evaluation of qualitative data. Statistical significancy was set at $p < 0.05$.

Results

189 patients with the diagnosis of ICD code in Table 1 or Table 2 were questioned, 135 the patients met the criterias of ILI. Three of the patients’ samples

Table 2. International Classification of Diseases Code version 10 (ICD 10).

Patients aged between 0 - 5 years	International Classification of Diseases Code version 10 (ICD 10)
Acute upper or lower respiratory disease	J00-J06, J20-J22
Dyspnea, breathing anomaly, shortness of breath, tachypnea	R06.0, R06, R06.9, R06.3, R06.00, R06.09, R06.83, R06.02, R06.82, R06.2, R06.89
Asthma	J45.2-J45.22, J45.9-J45.998, J44-J44.9
Pneumonia and influenza	J09-J18
Heart failure	I50-I50.9; I51.4
Myalgia	M79.1
Altered consciousness, convulsions, febrile convulsions	R40.20, R40.4, R40.0, R40.1, R56.00, R56.01
Fever or fever unknown origin or non-specified	R50, R50.9
Cough	R05
Gastrointestinal manifestations	A09.0; A09.9
Sepsis, Systemic inflammatory response syndrome	R65.10, R65.11, R65.20, A41.9

were transported in the wrong conditions for this reason these three patients removed out of the study.

A total of 132 hospitalized children with ILI between December 2013-May 2014 and December 2014-May 2015, (59% male, 41%female) were enrolled in this study. According to age distribution, 37 patients (28%) were aged <1 year, 80 patients (61%) were aged between 1 to 3 years, and 15 patients (11%) were aged > 3 years. A history of vaccination with the seasonal influenza vaccine was present in 11 patients (8%), 11 patients (8%) in 2012-2013 and 2013-2014 seasons, respectively. At least one respiratory virus was founded to be positive by RT-PCR in 78 (59%) patients, influenza A (H3N2) was detected in only 8 (6%) patients. In 54 (41%) patients samples were negative for any respiratory viral pathogens, on the other hand in 70 (53%) patients, one of other respiratory virus was detected other than influenza A.

The respiratory viral pathogens detected in decreasing rates were; RSV (n = 46, 35%), HCoV (n = 10, 7.5%), adenovirus (n = 7, 5%), rhinovirus (n = 6, 4.5%), HMPV (n = 5, 4%), Influenza B (n = 4, 3%) ve HBoV (n = 2, 1.5%). In 10 patients, coinfection was detected, however none of them was with H3N2. Seven of the coinfections were related to influenza RSV virus associated with the following single respiratory viruses: HCoV43 in two cases, HCoV229 in two cases, adenoviruses in one case, rhinovirus in one case and HMPV in one case. The other three coinfections were caused by: HCoV229 and adenovirus, HCoV229 and HMPV, HCoV 43 and influenza B.

In H3N2 (+) group, age older than three years (p = 0.006), asthma history (p = 0.015) and chronic lung diseases (p = 0.0001) were found to be risk factors. On the other hand in patients infected with any other virus

except Influenza A female gender was found to be a risk factor (p = 0.012). There were no statistically significant differences for birth weight, gestational age, duration of breastfeeding, admission to hospital during the last three months, history of vaccination with influenza, immunocompromised status, malignancy, renal insufficiency nor neuromuscular disease between the groups. Demographic characteristics and risk factors of children with ILI are shown in Table 3. No mortality was observed during the study.

Discussion

In this study, firstly we aimed to determine the prevalence of influenza A virus and epidemiological characteristics of patients with the diagnosis of ILI during the 2013-2014 and 2014-2015 influenza season. Secondly, we also investigated other respiratory viruses in these patients group. In our study, we found influenza A (H3N2) virus positivity in patients as 6%. In our country multicenter epidemiologic studies showed that, in 2005-2006, 2006-2007, 2008-2009 and 2011-2012 influenza season, H3N2 was the isolated type of influenza A, in 2007-2008, 2009-2010, 2010-2011 H1N1 was the predominant isolated type [16]. As far as we know, our study is the latest study to determine the prevalence of influenza A which has found H3N2 as the dominant type. There is no data of epidemiologic study of influenza in 2012-2013 season in our country, for this reason we don't know the dominant type of influenza for that period. We have found the overall vaccination for influenza was 8% both in 2012-2013 and 2013-2014 seasons. The influenza vaccination is not part of the routine immunization programme of our ministry of health and many parents prefer their children not to be vaccinated against influenza. This can be attributed to

Table 3. Comparison of Demographic Characteristics and Risk Factors between children with H3N2-positive, other virus detected and not detected presenting with Influenza-Like –illness.

Variables	H3N2 positive (n=8)	Other virus detected (n=70)	Not virus detected (n=54)	P value	
Age	< 1 year	0	17 (24)	20 (37)	0,028
	1 – 3 years	5 (62.5)	44 (63)	31 (57.4)	
	> 3 year	3 (37.5)	9 (13)	3 (5.5)	
Gender	Male	6 (75)	33 (47)	39 (72)	0.012
	Female	2 (25)	37 (53)	15 (28)	
Breastfeeding	6 (100)	70 (100)	49 (96)	0,238	
Chronic lung diseases	1 (14)	0	0	0,0001	
Asthma status	1 (14)	1 (1.4)	0	0,015	
Influenza vaccination status between 2012-2013	1 (12)	5 (7)	5 (9)	0.830	
Influenza vaccination status between 2013-2014	1 (12)	5 (7)	5 (9)	0.714	

the socioeconomic status of citizen, parents thinking that if it was necessary it should be in our routine immunization programme, and parents worrying about side effects.

In our study, 28% of hospitalized children were under 1 year, 61% were 1 to 3 years and 11% were older than three years. H3N2 was found to be significantly higher in children older than 3 years when compared to the virus negative group and children infected with any other viruses ($p = 0.028$). On the other hand, 5 of 8 patients infected with H3N2 were aged between 1 to 3 years. Vandepitte *et al* reported that, older age, having an underlying co-morbidity and a history of being premature were risk factors for influenza infection in logistic regression analysis [17]. In another study, Dalziel *et al* showed that a history of chronic lung disease, history of cerebral palsy / developmental delay are significantly associated with severe infection in children with ILI [18]. Because of the low frequency of H3N2 positivity in our study, it was difficult to evaluate risk factors in this study group.

The overall detection of at least one respiratory virus was 59% in our study. In one of the most recent studies, Wang *et al.* found at least one respiratory virus as 14.5% in hospitalized children with respiratory infection [19]. On the other hand, in the study of Kamikawa which was conducted in Brazil, at least one respiratory viruses were detected in 74% in outpatient children with the symptoms of common cold [20]. In two different studies from our country, at least one respiratory virus was found to be in children with ARTIs and ILI were 37% and 52%, respectively [21,22]. We found some differences in our study which may introduce some bias. Firstly, all the children in our study were hospitalized and this may be associated with severe symptoms, secondly the pharyngeal swabs were obtained during the two peak subsequent influenza season which may be associated with increased diagnostic rates and thirdly, we investigated a different variety of viruses to others.

RSV is one of the most common causes of acute respiratory infection in children under two years. In our study, RSV positivity was 35% in our hospitalized children with LRTI similar to other studies conducted in our country [23,24]. There were ten coinfections in our study and RSV was also the most common in coinfection. Studies showed different results about prognosis of coinfections, some of them reported that coinfections are associated with worse prognosis, however some of them found no significant difference between coinfections and infections with single virus [25,26]. In our study, there was no significant

difference of prognosis between coinfections with RSV and single virus infections.

One of the limitation of this study is, we did not record the clinical findings of the patients group. Because of low frequency of H3N2 positivity and vaccination rates, we could not determine the efficacy of influenza vaccination.

Conclusion

We found low rates of Influenza A in hospitalized children with ILI and low rates of vaccination with Influenza in our region. Different types of viruses with a wide range can cause ILI and hospitalization in children. We show that RSV is still one of the most common virus attributable to respiratory tract infections. Because of a lack of data showing the frequency of Influenza A in 2013-2014 and 2014-2015 season in our country, this study has importance because we show H3N2 predominance for those seasons.

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