Influenza and respiratory syncytial virus morbidity among 0-19 aged group in Yunus Emre Health Center

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The objective of the study was to determine the morbidity of influenza and respiratory syncytial virus (RSV) infection in the 0-19 years of age group with influenza-like illness among the outpatient cases. From 20 January to 31 March 2003 a total of 123 subjects with upper respiratory tract infection attended Yunus Emre Health Center. Ninety-one subjects fit the case definition of influenza-like illness, which consisted of acute fever of more than 38°C, cough, and sore throat. After obtaining their consent, nasal swabs were taken for isolation of influenza and RSV. Of these, 10 were influenza A virus, 6 were influenza B virus and 20 were RSV. All of influenza virus A was typed as subtype H3N2. The rates of influenza virus among 5-9 and 1-4 years of age groups and of RSV among 1-4 years of age group were high. The average number of absentee days of schoolchildren with influenza was 3.33 days and of those with RSV infection was 1.43 days; this rate was calculated as 2.25 days for the influenza-like illness. Continuous surveillance and influenza vaccination for target groups are recommended for beneficial effects of reducing influenza morbidity and mortality in the community.

Key words: influenza, respiratory syncytial virus (RSV), morbidity, health center.

Influenza is a highly contagious acute respiratory infection that has caused epidemics and pandemics of human diseases for centuries. The impact of influenza epidemics varies every year depending on the virulence of the circulating virus strain, insufficiency of prior immunity to the epidemic virus in the population and the intensity of exposure to the virus. The epidemic results in substantial morbidity and mortality every year[1].

The clinical presentation of influenza in children varies in different age groups, but in general, it is associated more commonly with a febrile illness than other respiratory viruses[2].

Respiratory syncytial virus (RSV) was identified in all age groups diagnosed with influenza-like illness; RSV was the dominant pathogen isolated from individuals in the community in winter[3]. It is best known for its tendency to cause bronchiolitis in infants and has been associated with substantial morbidity and mortality in children[4]. RSV epidemics often overlap with influenza epidemics[5]. However, rates of influenza and RSV infections among those with influenza-like illness are difficult to estimate because the infections must be identified virologically.

The present study was designed to determine the morbidity of influenza and RSV infection and the proportion of influenza and RSV infection in the group aged 0-19 years with influenza-like illness among the outpatient cases using virological isolation techniques.

Material and Methods

Yunus Emre Health Center has provided primary health care services since 1980 in Altındağ, Ankara. The population was 34,698 according to the health center's records in 2003, the year the study was conducted. The average

socioeconomic level was low-to-moderate. More than half of the population is covered by social security.

From 20 January to 31 March 2003 a total of 123 persons aged between 0-19 years attended Yunus Emre Health Center for upper respiratory tract infection. Symptoms such as acute fever of more than 38°C, cough, and sore throat were used for the case definition of influenza-like illness. The patients demonstrating the three symptoms were accepted as having influenza-like illness. Ninety-one of the 123 subjects fit the case definition and were included in this descriptive study. Informed consent was received from either the subjects or their parents, if the subject was younger than 18 years. The subjects with influenza-like illness were interviewed. After their consent, nasal secretion swabs were taken for isolation of influenza and RSV. The swabs were placed in transport medium at 4°C and sent to the Microbiology Department, Virology Unit, of Istanbul Medical Faculty, in 24 hours under the cold chain condition.

Laboratory Diagnosis of Influenza Virus and RSV

Materials received were centrifuged after stirring. Supernatants separated were used for isolation of influenza virus on Madin-Darby Canine Kidney (MDCK) cells and immunocapture ELISA, while cell pellet was used for direct IFA examination.

Slides prepared using cell pellets were stained with specific antibodies (DIF) against RSV (Bio-Rad, Redmond, USA) and influenza virus types A and B (Dako, Cambridgeshire, England) and examined with fluorescence microscope.

Cell cultures of influenza virus were performed simultaneously as classical culture and shell vial. For cell culture, monolayer MDCK cells were washed with serum free medium and inoculated with 100 μL of material. After the addition of medium containing trypsin-TPCK, cells were incubated in 5% CO_2 at 35°C for 5 to 7 days. Supernatants collected were used for hemagglutination (HA) and inhibition of hemagglutination (IHA) tests.

For immunocapture ELISA test, plates were sensitized using hyperimmune serums of rabbit against influenza virus type A (H_1N_1 and H_3N_2) and type B in carbonate-bicarbonate buffer system (pH 9.6). After adding hyperimmune serums, plates were incubated at +4°C for one night and washed with phosphate buffer solution (PBS) and tween 20.

Biotin conjugated anti-mouse antibody (Vector Laboratories, USA), avidin peroxidase conjugated (Vector Laboratories, USA), and chromogen substrate (Dynex, United Kingdom) were obtained commercially. Monoclonal antibodies of rabbit and mouse and reference serums of ferret specific to influenza virus types A (H_1N_1, H_3N_2) and B were kindly provided by the Institute Pasteur in Paris, France.

One hundred μl of material, influenza virus types A (H_1N_1 and H_3N_2) and B as positive controls and PBS as negative control were added into wells of plate. After incubation at 37°C for 1 h, the plates were washed with PBS and tween 20 and monoclonal antibodies of mouse specific to influenza virus types A and B were added and incubated. Biotin conjugated anti-mouse antibody was added after washing. When the plates were incubated and washed, conjugated avidin peroxidase was added.

Following incubation of the plates at 37°C for 10 min and washing, chromogen substrate was added. After reaction was stopped using 1 M H_3PO_4 solution, absorbance was read at 450 nm.

For HA test, PBS was added into the wells of a U bottom plate. Supernatants from cell culture were added into the first well of the first column and two-fold dilutions were made. After 0.5% erythrocyte suspension from guinea pig was added and incubated for 1 h at laboratory temperature, HA observed was recorded.

For IHA test, PBS was added into the wells of a U bottom plate. Reference serums from ferret specific against each virus type (receptor destroying enzyme treated and diluted in PBS and sodium azide) were added into the first well of the first column and two-fold dilutions were made. Supernatants from cell culture were diluted with PBS and sodium azide to the concentration of 4 HA unit and added to each of the wells. After incubation for 30 min at laboratory temperature, 0.5% erythrocyte suspension from guinea pig was added into the wells. Plates were incubated for 1 h at laboratory temperature. Virus type was determined according to the IHA observed.

Results

From a total of 1,445 patients, 123 (8.5%) had upper respiratory tract infection. Nasal swabs were obtained from 91 cases of influenza-like illness. The number of patients who attended the health center and the number of cases with upper respiratory illness and influenza-like illness diagnoses are presented in Table I. Upper respiratory tract illnesses peaked between 24-31 March and between 27 January 2 February 2003. Between 3-16 March 2003, all cases with upper respiratory tract illnesses fit the case definition.

As can be seen in Table III, influenza virus was isolated from two patients in the 0 age group. The influenza virus isolation rate was the highest among those aged 5-9 (13.6% for influenza A, 9.1% for influenza B), while among those aged 1-4, influenza A and influenza B were isolated at the same rate (10.7%). The isolation rates among the 1-4 and 5-9 years of age groups were not statistically significant. Influenza virus A and B were isolated together from two children aged 1-4. RSV had the highest isolation rate among the 1-4 years of age group.

Table I. Distribution of the Number of Patients Attending, and of Upper Respiratory Tract and Influenza-Like Illnesses by Weeks (Yunus Emre Health Center, 20 January-31 March 2003)

Consultation Date (2003)	Number of patients	Upper respiratory tract illness		Influenza-like illness	
	#	#	%(1)	#	%(2)
20-26 January	180	16	8.9	10	62.5
27 January – 2 February	133	17	12.9	14	82.3
3–9 February	153	12	7.8	8	66.7
17-23 February (3)	154	4	2.6	0	0.0
24 February – 2 March	130	8	6.2	3	37.5
3-9 March	167	14	8.4	14	100.0
10-16 March	182	12	6.6	12	100.0
17-23 March	178	16	9.0	14	87.5
24-31 March	168	24	14.3	16	66.7
Total	1445	123	8.5	91	74.0

1. Percentages were calculated according to the number of the patients.
2. Percentages were calculated according to the number of upper respiratory tract illnesses.
3. No one attended the health center between 10-16 February 2003 (closed for the holiday).

The distribution of influenza A and B virus and RSV is presented in Table II. Influenza virus and/or RSV were isolated from 32 (35.2%) of 91 influenza-like illness cases. The number of cases from which only RSV was isolated was 18 (56.3%). The total number of cases from which influenza virus was isolated was 14 (8 influenza A, 3 influenza B, 1 influenza A and B, 1 influenza B and RSV, 1 influenza A, B and RSV). All influenza virus A isolates were typed as subtype H3N2.

There was no significant difference in the isolation rate of influenza virus A between males (10.6%) and females (11.4%), while influenza virus B isolation rate was higher in males than females. Influenza A and B were found together in two children (1 M/1 F).

In the present study, the period between the onset of the symptoms and the time of the nasal swab was not statistically significant for the isolation of influenza viruses and RSV.

Table II. Distribution of Influenza Virus A and B and RSV

Virus	Number of patients	%
RSV (only)	18	56.3
Influenza A (only)	8	25.0
Influenza B (only)	3	9.4
Influenza A + Influenza B	1	3.1
Influenza A + Influenza B + RSV	1	3.1
Influenza B + RSV	1	3.1
Total	32	100.0

Table III. Characteristics of the Subjects by Isolated Agents

	Influenza-like illness %[a](#)	Influenza A (H_3N_2 antigen) %[b](#)	Influenza B %[b](#)	RSV %[b] (#)
Age groups				
0 (n:26)	61.5 (16)	12.5 (2)	--	18.8 (3)
1-4 (n:33)	84.8 (28)	10.7 (3)	10.7 (3)	35.7 (10)
5-9 (n:32)	68.8 (22)	13.6 (3)	9.1 (2)	22.7 (5)
10-14 (n:18)	66.7 (12)	--	8.3 (1)	27.8 (5)
15-19 (n:14)	92.9 (13)	15.4 (2)	--	23.1 (3)
Gender				
Male (n:64)	73.4 (47)	10.6 (5)	10.6 (5)	29.8 (14)
Female (n:59)	74.6 (44)	11.4 (5)	2.3 (1)	27.3 (12)
Status				
Pre-school (n:69)	59.4 (41)	14.6 (6)	9.8 (4)	31.7 (13)
Student (n:49)	96.0 (47)	8.5 (4)	4.3 (2)	25.5 (12)
Other (n:5)	60.0 (3)	--	--	33.3 (1)
Total (n:123)	74.0 (91)	10.1 (10)	6.7 (6)	28.6 (26)

[a] Percentages were calculated according to the number of upper respiratory tract illnesses.
[b] Percentages were calculated according to the number of the influenza-like illnesses.

Influenza virus A and B infection was less frequent among schoolchildren than among pre-school children. However, influenza infections did result in student absenteeism. For influenza cases, the average absentee rate was 3.33 days, while this rate due to RSV infection was 1.43 days and for influenza-like illness was 2.25 days. Although the average rate of absenteeism was not statistically significant among the cases, influenza infections may influence the duration of absenteeism. Students were absent 16 days due to influenza, 12 days due to RSV infection and 46 days due to influenza-like illness, for a total of 74 absent days. The results suggest that influenza, RSV infection and influenza-like illness can contribute considerably to absenteeism.

The distribution of influenza virus A and B, RSV and influenza-like illness cases by onset of the symptoms is shown in Figure 1.

In January and February 2003, the number of influenza-like illness cases was similar to the number of influenza and RSV cases. However,

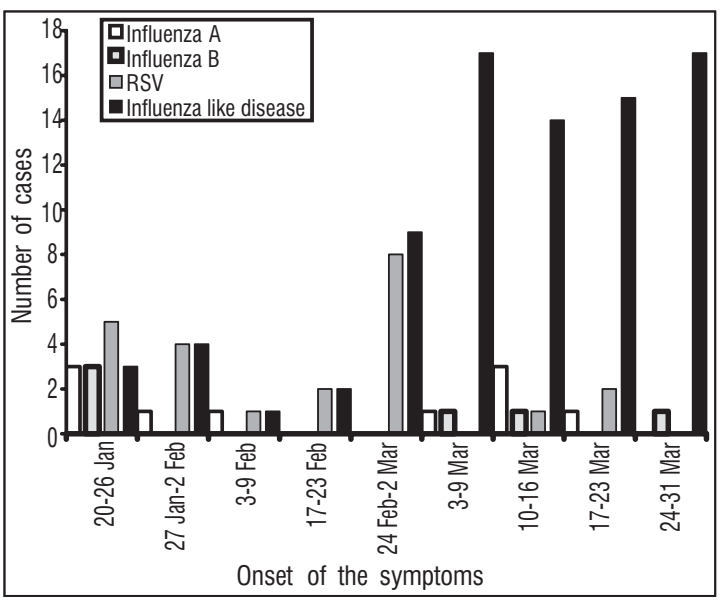

Fig. 1. The distribution of influenza virus A and B, RSV and influenza-like illness cases by onset of the symptoms.

while the number of influenza-like illness cases increased in March 2003, isolation rates of RSV and influenza viruses were decreased among the influenza-like illness cases, throughout March 2003. The peak of influenza virus and RSV detection did not coincide with the peak of influenza-like illness, i.e. between 20 January-31 March 2003.

Discussion

During the nine weeks between 20 January – 31 March 2003, 1,445 people aged 0-19 years attended the Health Center for various reasons, and 123 (8.4%) of them were diagnosed to have upper respiratory tract diseases: 91 cases as influenza-like illness. In the outpatient clinic, physicians consulted 5.8% of all the attendants for influenza-like illness. According to surveillance results in England, 4.9% of the population consulted a general practitioner with influenza during an epidemic[2].

In this study, 91 specimens were tested for influenza viruses, of which 16 (17.6%) were positive. Of these, 10 (62.5%) were influenza virus A (all were H3N2 antigenic type) and 6 (37.5%) were influenza virus B. Both influenza virus A and B were identified in samples taken from two cases (1 male, 1 female). As a result, 16 influenza viruses were isolated from 14 cases. The data suggested that influenza A and B viruses co-circulated in that period but influenza A virus was likely predominant. In Asia, Europe and North America, influenza A and B viruses co-circulated during October 2002–May 2003. In Europe and Asia the majority of influenza A viruses subtyped were A (H3N2), similar to the results found in the present study[6].

The influenza infection rates in our study were similar between boys and girls. The influenza viruses were isolated highest among those aged 5-9 years (13.6% for influenza A, 9.1% for influenza B) and 1-4 years (10.7% for influenza A, 10.7% for influenza B). The isolation rates were similar between those age groups. However, influenza virus was isolated from only one subject among the group aged 10-14 years, the age group with the lowest isolation rate. Cases among schoolchildren usually peak in the period of an influenza epidemic[7]. Therefore, the children play an important role in the introduction and spread of influenza virus in infants, adults and elderly in the household and in the community[8]. After the introduction of infection into households, secondary attack rates among family members averaged 24% for A/H3N2[2]. The risk of influenza and RSV infection can elevate in elderly people and other members of the family with associated chronic illnesses.

In a laboratory-based surveillance study, RSV was determined in 18,418 of 128,117 specimens tested in the 1998-1999 study season, with an isolation rate of 14.3%[9]. In this study, 20 samples were identified as containing RSV, giving an isolation rate of 28.6% in all patients. RSV isolated from nasal swabs was the more frequent viral pathogen versus influenza viruses, and accounted for 14 cases (15.4%). RSV infections were present in every age group but were higher in the group aged 1-4 years. Studies showed that RSV was identified in all age groups diagnosed with influenza-like illness in the community or household. Together, influenza virus and RSV accounted for 35.1% of pathogens identified in the samples taken from cases of influenza-like illness over the study period. Therefore, it is an important pathogen contributing to the burden of influenza-like illness in the entire community during winter[10,11].

It has been recommended that absenteeism due to influenza-like illness could be a useful indirect measure of morbidity of influenza[2]. Among the school-aged group, the average absenteeism related to influenza was found as 3.3 days. When we compared influenza-positive children with others who had influenza-like illness, the children with influenza were found to have a longer duration of absenteeism. In one survey, it was shown that influenza has multiple effects, such as school absenteeism. The duration of absenteeism was 5.1 days among influenza-positive children, versus 4.25 days among influenza-negative children[12]. In the Seattle area during the 2000-2001 influenza season, 63 missed school days were estimated for every 100 children[13]. In Russia, missed school rate was found higher, as 79 days for every unvaccinated child[14]. In our group, we observed 16 missed school days for 49 schoolchildren, which suggests lower absenteeism rates compared to the above-mentioned surveys[13,14]. This could be at least partially explained by the health center-based nature of our data versus the population-based nature of earlier studies. Only schoolchildren attending Yunus Emre

Health Center are presented here. Therefore, no comment can be made regarding the days of school missed by children with influenza who did not present to any health facility, who presented to another (private or governmental) facility, or who visited private physicians' offices.

As can be seen in Figure 1, influenza virus A, B and RSV were co-circulated throughout the nine weeks. At the end of February 2003, RSV peaked and influenza-like illness similarly increased. In March 2003, influenza and RSV decreased, while influenza-like illness remained at a high level. The studies suggest that the occurrence of influenza-like illness varies year to year, and the relative proportions of influenza and RSV have changed over the seasons[15].

Death associated with influenza, RSV and influenza-like illness did not occur in the present study. However, influenza causes roughly 19,000 deaths and 114,000 hospitalizations per year in the United States[16]. Over 90% of influenza-related deaths occur in the elderly. Vaccination against influenza has been found to reduce all-cause mortality by 35-59% during an influenza epidemic[17]. Although influenza-related deaths are uncommon in children 6 to 23 months of age, children with certain chronic conditions and healthy children younger than 24 months were hospitalized for influenza and its complications at high rates, similar to those observed in the elderly[18,19]. Within the 0-4 age group, hospitalization rates are highest among children aged 0-1 years and are comparable to rates reported among persons ≥65 years[20,21]. In Japan, results of a prospective, Internet-based study showed that influenza respective vaccine efficacy for one- and two-dose regimens was 54.0% and 79.8% for laboratory-confirmed influenza in the 0-15 years of age group[22].

In one study, children who had received the influenza vaccine had significantly fewer respiratory tract infections, and antibiotic and antipyretic prescriptions than did the unvaccinated children, but the effectiveness on reducing hospital stays was not significantly different between the vaccinated and unvaccinated children[15].

Economic analysis also showed that routine vaccination of high-risk children against influenza would be cost saving for society[23].

In conclusion, in individuals diagnosed with influenza-like illness, there is a substantial potential for confusion between illnesses caused by influenza and/or RSV infection. Linked clinical-virological surveillance for influenza and RSV should be carried out in Turkey. This approach can provide early warning of influenza and/or RSV infection epidemics. This study reaffirms the need for continuous surveillance of influenza and RSV as an important public health issue. The studies have shown that influenza vaccination has beneficial effects of reducing influenza diseases and mortality in large communities[24]. It also raises questions about a rational vaccination policy for influenza to increase herd immunity in Turkey.

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