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A SURVEY OF RESPIRATORY SYNCYTIAL VIRUS IN CHILDREN IN THREE EDUCATIONAL AND THERAPEUTIC PEDIATRIC CENTERS IN TEHRAN

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ABSTRACT

Nasopharyngeal swab samples from patients with an acute flu-like illness were evaluated for the presence of respiratory syncytial virus (RSV) from October 1996 to March 1998. The relative frequency and seasonal distribution of RSV was assessed. In addition, the virus correlated with specific clinical signs and symptoms.

During the study, 268 samples were collected from children under the age of 14 years in Virus Transport Medium (VTM) in three educational and therapeutic pediatric centers in Tehran by participating medical practitioners. The specimens were tested for RSV by virus isolation and direct immunofluorescense (DIF) technique in the virology section of Pasteur Institute of Iran. Respiratory syncytial virus was detected from 33 samples (12.3%). In this study the highest rate of RSV was found in children less than 1 year of age (19.5%), but the male to female ratio in patients was approximately equal (1:1). RSV infections peaked in the early winter, as 85% were detected from December to March. There was a statistically significant difference between age and RSV infection ($p<0.001$), but the difference between sex and season with infection was not statistically significant ($p>0.05$). One of the most common clinical signs and symptoms in patients was bronchiolitis, which was observed in 48.5% of subjects infected with RSV.
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INTRODUCTION

Data from the World Health Organization (WHO) show that acute lower respiratory tract infections are a major cause of morbidity and mortality in children in developing countries.[1,2] Viruses such as the respiratory syncytial virus (RSV), influenza virus, para-influenza virus and adenovirus play in important role in respiratory infections in children.[3,4] However there are few reports on the epidemiology of viral respiratory infections in developing countries, because of the limitations in diagnostic technology. RSV is the most common cause of acute respiratory infections (ARI) in infants and young children in temperate regions,[5,6] usually outranking all other microbial pathogens as the cause of bronchiolitis and pneumonia in infants under 1 year of age. Respiratory syncytial virus accounts for about half of bronchiolitis cases and one-fourth of pneumonias in infants. It is estimated to cause about 4500 deaths per year in the USA.[2] Respiratory syncytial virus is transmitted via large droplets, so spread can occur by contact with contaminated hands or surfaces.[2,4]

MATERIAL AND METHODS

An epidemiological study on acute lower respiratory infections (ALRI) with respiratory syncytial virus (RSV) was carried out in children under 14 years old in Tehran from October 1996 to March 1998.

Patients and specimens

Patients were recruited from three educational and

therapeutic pediatric centers in Tehran. Clinical data on each patient was collected using standard questionnaires including sex, age, season, clinical manifestations, clinical diagnosis and complications.

The diagnosis of ALRI was based on the presence of bronchiolitis, cough, fever and one or more of the following: tachypnea, chest retractions, rales or stridor. Two nasopharyngeal swabs (NPS) were collected from each child for viral isolation and direct immunofluorescence (DIF) during the study period.

Specimens were collected by swabbing the posterior pharynx via the nostril. One of the swabs was immediately placed into 3 mL chilled transport medium MEM (minimum essential medium, with 3 percent bovine serum albumin, and 500 µg of penicillin (5,000,000 U/mL) and 50 µg streptomycin per mL and 2µg of amphotericin B/mL) and taken to the laboratory within 2 hours of collections. The other swab was rolled directly on three slides to obtain cell smears. Slides were air dried, fixed in cold acetone, and then kept at -70°C until staining.

Immunofluorescence technique

The acetone-fixed cells were stained by direct immunofluorescence (DIF) method with the use of fluorescein isothiocyanate labelled monoclonal antibodies to RSV (Dako-Denmark). Each slide was covered with two drops of the specific monoclonal antibody and incubated in a humidified chamber at 37°C for 15 min, then washed with PBS for 5 min and allowed to dry. Before reading, 2 drops of mounting fluid were placed on the slide and covered with a cover slip. Slides were evaluated independently by two trained personnel using a UV light microscope.

The specimens were considered adequate for evaluation when there were more than eight cells per field at 200× magnification. HEP-2 cells infected with RSV served as positive controls while non-infected cells served as negative ones.[9,10]

Virus isolation (VI)

HEP-2 cell monolayers grown in 25 mL culture flasks were used for the isolation of RSV. Cells were propagated in MEM (Gibco) supplemented with 10 percent fetal calf serum. Specimens in transport medium were vortexed vigorously and 0.5-1 mL of each sample was inoculated in to one cell culture flask. After a 15 min absorption period, the cultures were reefed with MEM+3 percent fetal calf serum and incubated at 36°C. Monolayers were examined daily for 10 days for the presence of cytopathic effect (CPE). At the end of the incubation period all flasks were processed for DIF regardless of the presence of CPE and were tested on the day when there was 50-75 percent cell degeneration. Cells were processed for DIF by scraping part of them into 0.2mL of MEM, centrifuged at 1500 rev/min, and resuspended into PBS. Cells were spotted on multiwell slides, allowed to dry, fixed with acetone, and stained with virus-specific monoclonal antibodies.[9,10]

RESULTS

During the 18 month study period, nasopharyngeal swabs were collected from 268 children with ARI from October 1996 to March 1998. Of a total of 268 swab specimens from infants and young children investigated for respiratory syncytial virus (RSV) in three educational and therapeutic pediatric centers in Tehran, RSV was detected from 12.3% (33/268) of samples.

Table I. Number of RSV isolates and isolation rates in children by age group.

Age (year)	Number of samples	Number of isolates	Rate (%)
<1	82	16	19.5
1-2	72	9	12.5
3-5	63	5	7.9
6-14	51	3	5.9
Total	268	33	12.3

The data show that the majority of RSV infections was in infants less than 1 year of age (19.5%). There was a significant statistical difference between age and RSV infection ($p<0.001$) (Table I). The male to female ratio in patients with RSV was approximately equal (1:1). The results of this study show that the rate of RSV de-

Table II. Seasonal (months) distribution of RSV isolates and isolation rates in Tehran from October 1996 to March 1998.

Season (Months)	Number of Samples	Number of Isolates and Rate (%)
JAN	46	9 (27.3)
FEB	54	10 (30.3)
MAR	39	6 (18.2)
APR	22	1 (3)
MAY	15	0 (0)
JUN	10	0 (0)
JUL	8	0 (0)
AUG	6	0 (0)
SEP	10	1 (3)
OCT	12	1 (3)
NOV	18	2 (6.1)
DEC	28	3 (9.1)
Total (100)	268	33 (100)

Table III. Percentage of clinical symptoms observed in 268 patients with ARI.

Clinical observation	Number of ARI cases (%)	Number of RSV cases (%)
Respiratory symptoms:		
Clear nasal secretions	205 (80.2)	25 (75.7)
Purulent nasal secretions	35 (13.4)	4 (12.1)
Cough	232 (86.6)	29 (87.9)
Physical findings:		
Temp.<38.5	60 (22.4)	7 (21.2)
Temp. between 38.5-39.5	119 (44.4)	15 (45.4)
Temp.>39.5	89 (33.2)	12 (36.4)
Pharyngitis	117 (43.6)	15 (45.4)
Otitis	86 (32.1)	11 (33.3)
Bronchiolitis (rales or weezing)	124 (46.3)	16 (48.5)
Pneumonia	19 (7.1)	3 (9)
General symptoms:		
Emesis	42 (15.6)	5 (15.1)
Diarrhea	28 (10.4)	3 (9)
Abdominal pain	19 (7.1)	2 (6)
Headache	47 (17.5)	6 (18.1)

tection was not equal during the 18 month surveillance period, because more than 85% (28/33) of RSV cases were detected in the winter (December to March) (Table II). There were no hospitalizations for RSV infection from May to August. In the present study the most common clinical signs and symptoms in patients with RSV infection were fever, cough and bronchiolitis; 36.4% of patients showed a temperature > 39.5°C, 87.9% of them cough, and 48.5% had bronchiolitis (Table III).

DISCUSSION

In most parts of the world, viral respiratory tract infections are among the most common causes of morbidity and mortality, especially in children under the age of 5 years old.[11,12]

RSV is one of the main causes of childhood pneumonia worldwide, and seems to have a seasonal pattern.[13] Immunity to RSV infection is incomplete, and repeated infections throughout life are common.[14]

In this study, laboratory diagnosis was attempted by using two methods (VI and DIF) for virus detection (RSV).

Our results show that RSV was the most frequently detected pathogen (12.3%). This is in accordance with results from different parts of the world. Greenwood reported that RSV was one of the most important causes of acute lower respiratory infection in Gambia.[15] The distribution of RSV in the present study seems to be similar to those in developing countries and other tropical regions, and it is the predominant pathogen of respiratory tract infections in infants and young children.[16] RSV infection in small children is severe and life-threatening in some cases.[7,8] Most United States studies reported hospitalization rates for RSV infections of 1 to 20 per 1000 children less than 1 year of age.[17] In our study 19.5% of patients with RSV were children less than 1 year of age ($p<0.001$), a distribution similar to that reported by others.[12,16,18] In the present study bronchiolitis, cough and fever were the most common specific signs and symptoms in children with RSV infection, therefore the clinical features of these patients are similar to those previously described by Lina et al. in France.[19]

In temperate communities, RSV peaks during the winter months, whether in the southern or northern hemisphere.[20,21]

Our data indicate that the outbreaks of RSV infection were in the winter (December to March), so approximately more than 85% of RSV has been isolated in this season. These results are in accordance with the findings of Chew and colleagues[22] and Lina and colleagues.[19]

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REFERENCES

1. Glezen WP, Cough RB: Influenza Viruses, Viral Infections of Humans, Epidemiology and Control. 3rd edition, New York: Plume Publishing, pp. 419-50, 1991.
2. World Health Organization: Program for the control of acute

respiratory infections. Weekly Epidemiol Rec 68: 353-7, 1993.
3. Bulla A, Hitze KI: Acute respiratory infections: a review. Bull WHO 56: 481-98, 1978.
4. Walter EB, Shurin PA: Acute respiratory infections. In: Krugmans TI, (ed.), Infectious Diseases of Children, 9th edition, St. Louis: Mosby Year Book, pp. 329-76, 1992.
5. Saijo M, Ishii T, Kukubo M, Takimoto M, Takahashi Y: Respiratory tract and asthma attack in hospitalized children in North Hokkaida, Japan. Acta Pediatr Jpn 35: 233-7, 1993.
6. Popow-Kraupp FC, Kunz C, Huber E: Respiratory virus infection in hospitalized children in Austria 1979-1982. Infection 12: 185-9, 1984.
7. Disney ME, Sandiford BR, Cragg J, Wolff J: Epidemic bronchiolitis in infants. BMJ 1: 1407-11, 1960.
8. Heycock JB, Noble TC: 1230 cases of acute bronchiolitis in infancy. BMJ 2: 879-81, 1962.
9. Numazaki TM, Oshimat F, Ohmia PL, et al; A microplate method for isolation of viruses from infants and children with acute respiratory infections. Microbiol Immunol 31: 1085-95, 1987.
10. Ballew H, Forrester FT, Lyerla HC, Vellecaw M, Bird BR: Microsera neutralization in laboratory diagnosis of viral diseases. 5th edition, Atlanta, Georgia: Welfare Public Health Service, pp. 234-285, 1988.
11. Dowell SF, Anderson LJ, Gray HE jr, et al: Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. J Infect Dis 174: 456-62, 1996.
12. Monte AS, Sullivan KM: Acute respiratory illness in the community, frequency of illness and the agents involved. Epidemiol Infect 111: 145-60, 1993.
13. Avila MM, Carballal H, Rovaletti B, Elekian M, Cusminsky BA, Weissenbacher M: Viral etiology in acute lower respiratory infections in children from a closed community. Am Rev Respir Infect 140: 634-637, 1989.
14. Panuska JR, Merolla R, Rebert NA, Hoffman SP, Tsivitse P, Cirino NM, Silverman RH, Rankin JA: Respiratory syncytial virus induces interleukin 10 production by human alveolar macrophages. Suppression of early cytokine production and implications for incomplete immunity. J Clin Invest 96: 2445-2453, 1995.
15. Greenwood B: Epidemiology of acute lower respiratory influenzae, especially those due to *Haemaphilus influenzae* type b, in Gambia. West Africa J Infect Dis 165: 526-8, 1992.
16. Reyes M, Hedlund KO, Lorenzana I, Ehrnest A: Respiratory infection and iatrogenic diarrhea in Honduras and Elsalvador duing the 1991-1992 season. AMJ Trop Med Hyg 54: 260-4, 1996.
17. Glezen WP, Paredes A, Allisan JE, Taber LH, Frank AL: Risk of respiratory syncytial virus infection for infants from low income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 98: 708-15, 1981.
18. Doraisingham S, Ling AE: Pattern of viral respiratory tract infections in Singapore. Ann Acad Med Singapore 15: 9-14, 1986.
19. Macintosh K, McQuillin J, Reed SB, Gardner PS: Diagnosis of human coronavirus infection by immunofluorescence method and application to respiratory disease in hospitalized children. J Med Virol 2: 341-346, 1978.
20. Gilchrists AE, Torok TJ, Gary HE, Alexander JP, Anderson LJ: National surveillance for respiratory syncytial virus-United States. J Infect Dis 170: 986-90, 1994.
21. Thomas E, Margach MJ, Orvell C, Moriiaon B, Wilson E: Respiratory syncytial virus subgroup B dominance during one winter season between 1987 and 1992 in Vancouver, Canada. J Clin Microbiol 32: 238-42, 1994.
22. Chew FT, Doraisingham S, Ling AE, Kumarasinghe G, Lee BW: Seasonal trends of viral respiratory tract infections in the tropics. Epidemiol Infect 121: 121-128, 1998.