

Original Article

Accurance of Respiratory Syncytial Virus Infection in Children Referred to Kasra Hospital Diagnostic Laboratory during 2009-2011

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Abstract

Background and Aims: Respiratory syncytial virus (RSV) infection is common in infants and young children. In infants younger than one year old it may cause bronchiolitis and pneumonia which requires hospitalization. Accurate and rapid diagnosis of the disease will help proper treatment of the disease and prevents further complications.

Methods: Specimen taken from respiratory tract of sick children were processed and fixed for immunofluorescence staining. Monoclonal antibody conjugate specific for RSV was used and the specimens were examined by a UV microscope.

Results: A total of 132 samples examined, 40.1% were positive for RSV. The disease was mostly in children less than 6 months old. It occurred mainly during the winter months and there were no significant differences between the male and female children.

Conclusion: RSV infects many infants and children annually during the winter. The virus probably infects considerable number of children across Iran. Proper diagnosis of the disease will help in treatment and control of the disease.

Keywords: Respiratory syncytial virus (RSV); Monoclonal antibody; Immunofluorescence Staining

Introduction

The most important viral infection of the lower respiratory tract in infants and children is caused by respiratory syncytial virus (RSV) (1). For children younger than 1 year, serious RSV infection of the lower respiratory tract may require hospitalization for bronchiolitis or pneumonia (2, 3). In the united states, up to 126/000 infants are hospitalized each year because of severe RSV disease (4, 5). The RSV season in the united states, as acknowledged by the American Academy of Pediatrics (6), generally

extends from November through March with a peak in January or February (6, 7). In temperate weather regions, RSV epidemics occur annually in the winter months, whereas in tropical areas, the out breaks are associated with the rainy season (8, 9), and, in semi-tropical areas, the virus circulates mainly during autumn (10). During the RSV season, it is estimated that about 40% of children will develop a lower respiratory tract infection (11). Since there have been few reports of this infection in Iran over the last years (12), and most epidemiological data on RSV infection are drawn from studies in Canada, the United States, and Europe, we evaluated infection rates of respiratory syncytial virus infection in referred patients to the diagnostic laboratory of Kasra Hospital in Tehran. Data were collected from children up to 3 years of age, from November 2009 to March 2011 with symptoms

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suggesting lower respiratory tract viral infections.

Methods

Patients

Specimens from patients with respiratory tract infection were referred to the diagnostic laboratory of Kasra Hospital. Patients were one month to 3 years old with symptoms of mild fever, respiratory infection signs such as cough, coryza, sometimes bronchial wheezing. Along with the submission of specimen the patients identifications such as age, sex, disease duration and the date were recorded. Specimens were taken by physician or trained health personnel. They included either nasopharyngeal wash or swabs soaked in PBS which were applied to the nasal opening with careful rotation to scrape off surface epithelial cells. The swabs were placed in a tube containing 0.5ml of PBS. All the specimens including nasopharyngeal wash, auger suction and PBS from the washed swabs were centrifuged at 3000rpm for 5min to sediment the cells. The pellets were washed once with PBS and then suspended in 200 μ lit of PBS. Approximately 10 μ lit of the pellet suspension were placed on each well of the diagnostic slides. The slides were six wells or 12 wells. They were allowed to air dry at room temperature and stored at 4 $^{\circ}$ C no longer than twenty four hours (for immunofluorescence staining).

Table 1. Distribution of positive tests for RSV during the winter months in 132 admitted children to Kasra hospital.

Month	November	December	January	February	March	Total
Patients	14	36	35	21	6	112
RSV(+)	4	15	16	13	1	49

Immunofluorescence staining

The dried specimen slides were fixed in cold acetone at -20 $^{\circ}$ C for 10 min.

Following fixation time, slides were taken out from the acetone and, rinsed in PBS and drained at room temperature. Approximately 10 μ lit of monoclonal antibody to G protein of RSV virion conjugated to fluorescein were applied to each well containing specimen.

(The monoclonal antibody conjugate was obtained directly from Abott Company, U.S.A) The slides were incubated in a humidified chamber at 37 $^{\circ}$ C for 1hr. After the incubation period they were washed in PBS and mounted using glycerol buffer pH 8.2. The slides were examined within an hour by a UV microscope. Controls were similarly stained using anti rotavirus IgG conjugate.

Results

The specimens taken from patients during November 2009 to March 2011 were delivered to the diagnostic laboratory at Kasra Hospital. The patients had respiratory infection symptoms clinically suspected of RSV infection. The specimens were stained with the direct method of staining. The wells containing the specimens were examined completely and at least five cells in each well showing fluorescence were considered positive. Usually the cells containing RSV antigen showed bright green Fluorescence which most of the

Table 2. Frequency of positive cases based on age of children suffering from RSV infection.

Age group(months)	Number	RSV(+)	RSV(+)%
0-6	87	38	71.6
6-12	19	8	15
12-24	17	6	11.3
24-36	9	1	1.8
Total	132	53	100

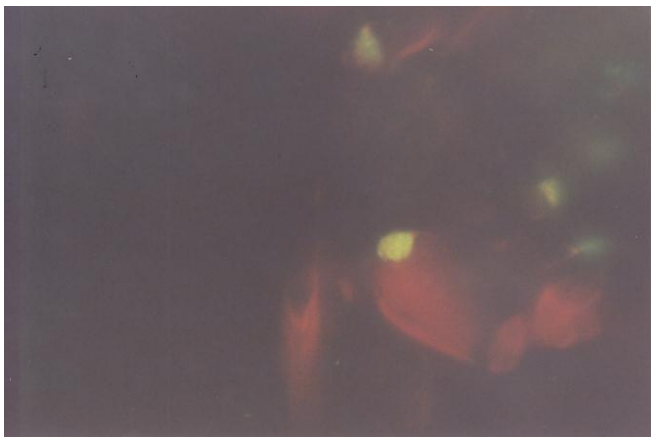


Fig. 1. Specimens containing respiratory epithelial infected cells were stained as described. Cells showing bright intracytoplasmic immune-fluorescence were positive.

time were located at one pole of the cell cytoplasm (Fig. 1) and occasionally in the form

of fine granules. The most infection rate was during the winter time. Out of the total of 132 patients 112 patients referred from Nov to March, 43.8% of them were positive for RSV (Table 1). The age of the patients varied from one month to three years old. Most of infections accrued in children during the first few month of age (Table 2). As it is shown, 71.6% of the positive cases were in children up to six months old and the least were in the two to three years old patients (1.8%). There were no significant differences between the male and female infection (Table 3) although the positive cases in male patients was slightly higher than the female, 58.4% male vs 41.5% of female.

Discussion

Respiratory syncytial disease is one of the most important respiratory infections of infants and

Table 3. Frequency of RSV positive cases based on sex of the patients.

Total		Sex			
Patients	Positive cases	Female		Male	
		Patients	RSV(+)%	Patients	RSV(+)%
132	53	60	41.5	72	58.4

young children (1, 2). Although it is common in earlier age but there have been several reports of outbreaks in old people at the old age care centers (1, 3). The disease may be mild and could be resolved after few days but in many cases it can cause severe bronchiolitis and become life threatening with some mortality (3). There are two strains of the virus causing the disease (14). The virus is an enveloped virus and very fragile, therefore it can be inactivated easily under normal environmental conditions. This susceptibility to inactivation makes it difficult to isolate the virus in tissue culture. However, under proper condition, the virus can grow in cell culture causing typical syncytial formation, characteristic of the cytopathic effect, causing by this virus. It has been reported that presence of calcium is required for syncytium formation (15). In the absence of calcium virus antigen can be detected in individual infected cells without cell fusion. In the present study the technique of immunofluorescence staining was used for detection of RSV infection in infants and young children. The technique is very specific, rapid and relatively easy to perform. We found that the disease occurs mainly in cold season. During the summer months the infection is very low. Most of the infections were detected in one to six month of age. The study showed that RSV infection is very common in children in Tehran and perhaps in Iran. It should be diagnosed accurately for proper treatment and should be diagnosed differentially from the bacterial and other viral infections.

References

- Glezen WP, Denney FW. Epidemiology of acute lower respiratory disease in children. *N Engl J Med*. 1973; 288:498-505.
- Glezen WP, Taber LH, Frank AL, Kasel JA: Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child*. 1986;140:543-546.
- Carbonell-Estrany X, Ruero J, and the IRIS study group. Hospitalization rates for respiratory syncytial virus infection in premature infants born during two consecutive seasons. *Pediatric Infect Dis J*. 2001;20:874-879.
- Shay DK, Holman RC, Newman RD. Bronchiolitis associated hospitalizations among US children, 1980-1996. *JAMA*. 1999;282:1440-1446.
- Sangare L, Curtis MP, Ahmad S. Hospitalization for respiratory syncytial virus among California infants: disparities related to race, insurance, and geography. *J pediatr*. 2006;149:373-7.
- Respiratory syncytial virus, In: Pickering LK, Baker CJ, Long SS, Mcmillan JS, eds. *Redbook: 2006. Report of committee on Infectious Diseases*. 27th ed. Elk Grove village, IL: American Academy of pediatrics; 2006:560-566.
- Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. *J Pediatr*. 2003;143:S 112-117.
- Cane PA. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol*. 2001;11:103-116.
- Nwantko MV, Dym AM, Shnit KE, Offer E, Omene JA. Seasonal variation in respiratory syncytial virus infection in children in Benin-city, Nigeria. *Trop Geogr Med*. 1988;40:309-313.
- Vieira SE, Stewien KE, Queiros DAO, Durigon EL, Torok TJ, Anderson LJ, Miyao CR, Hein N, Botosso VF, Pahl MM, Gilio AE, Ejzenberg B, Okay Y. Clinical patterns and seasonal trends in respiratory syncytial virus hospitalization in sao Paulo, Brazil. *Rev Inst Med trop sao Paulo*. 2001;43:125-131.
- Stensballe LG, Devasundaram JK, Simoes EF. Respiratory syncytial virus epidemics: ups and downs of a seasonal virus. *pediatr Infect Dis J*. 2003;22:521-32.
- Milani M. Respiratory syncytial virus infection among young children with acute respiratory infection *Acta Medica Iranica*. 2003;41:4-7.
- Falsey AR, Hennessey PA, Formica MA, et al. Respiratory syncytial virus infection in elderly and high-risk adults, *N Engl J Med*. 2005;352:1749-1759.
- Coggins WB, Lefkowitz EJ, Sullender WM. Genetic variability among group A and group B respiratory syncytial viruses in a children's hospital. *J Clin microbiol*. 1998; 36:3552-3557.
- Shahrabadi MS, Lee PWK. Calcium requirement for syncytium formation in Hep-2 cells by respiratory syncytial virus. *J clin Microbiol*. 1988; 26:139-141.