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Original Article

Respiratory Syncytial Virus Infection in Patients Referred to Kasra Hospital Laboratory during 2016-2019: A Continuous Study

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Abstract

Background and Aims: One of the main causes of severe respiratory infection in infants and young children is respiratory syncytial virus (RSV). The disease can also occur in adults and elderly individuals and clinically not to be differentiated from other viral respiratory infection. The disease causes bronchiolitis, and sometimes pneumonia in new born and young children which requires hospital care. To differentiate the disease from other respiratory infection and rapid treatment accurate laboratory diagnosis of the disease is necessary.

Materials and Methods: specimen taken from the sick children suffering from respiratory infection were processed and fixed on the slides. They were stained with fluorescein conjugated RSV specific antibody and examined by a UV microscope.

Results: From 141 patients attending the Kasra hospital laboratories 62 of them were positive, most of the infection occurred in children with in 6 month of birth. The rate of infection was higher in the month of January and February.

Conclusions: It seems that RSV infection is common in infants and young children which occur mostly during the cold season.

Keywords: Respiratory Syncytial Virus (RSV), Monoclonal antibody, Immunofluorescence staining

Introduction

One of the most severe respiratory diseases in infants and children is caused by the respiratory syncytial virus. The virus which was first isolated from a chimpanzee and later on identified to be identical to the virus isolated from a child suffering from pneumonia (1, 2). Soon after that it was determined that the virus was the major cause of bronchiolitis and pneumonia in infants and young children and was named respiratory syncytial virus (RSV) children (3-5).

The virus belongs to the family of paramyxoviridae order mononegavirales. Morphologically the virion is pleomorphic surrounded by a membrane with closely spaced spike projections. The viral genome is a single stranded with negative polarity (6).

The virus contains at least seven structural proteins which among these three of them (F.G.SH) are the surface proteins which are expressed on the surface of infected cells. The G and F glycoproteins play a role in production of neutralizing antibodies (7). The virus grows in variety of human cells but the most suitable cells for primary virus isolation are HEP-2 and Hela cells (8, 9). The virus infected cells show cytopathic effect which is manifested as cell fusion and syncytium formation. The F protein plays a major role in cell fusion and induction of syncytium (10). The infection is mostly

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among infants and children less than one year old but older children and adults in particular elderly people can become infected. The symptoms appear as common cold and mostly developed to bronchiolitis and pneumonia which might require hospitalization (11, 12). Most of the infection occurs during the cold season from mid fall to early spring but in tropical areas it can happen all the year around (13, 14). This is a continuous study following the previous report (15, 16) on the occurrence of RSV infection in children in Tehran which can be important to bring under consideration for prevention the spread of the disease.

Methods

Patients with respiratory infection suspected of RSV infection were referred to the diagnostic laboratory of Kasra hospital. Patients were from two weak to four years old. Some were hospitalized in the pediatric word of Kasra medical center but most of them were out patients who were referred to the laboratory. Almost all such individuals were suffering from respiratory infection with symptoms including fever, cough, sever nasal obstruction and coryza. Some of the patients had the sign of bronchiolitis and bronchial wheezing. For taking the samples, sterile tube containing one ml of buffered saline were used. Sterile cotton swabs were soaked in the saline and carefully inserted in to the nasal cavity or laryngeal area with slow rotation. In some cases pharyngeal washing and or auger suction were taken. The cotton swabs were washed in the PBS containing tube and the washing solution were delivered in to 1.5 ml microspin tubes and centrifuged at 5000 rpm for 5 minutes. The supernatant was carefully removed and the pellet was resusperded in 200 ml of H2O to be used for immunofluorescence staining.

Coating of glass slides with specimen

Twelve well glass slides were found to be suitable for coating. The minimum amount of the specimen (10μlit) could cover the entire well area and therefore, reducing the amount of reagents to be used for the test. The slides were cleaned with an aceton soaked swab, rinsed in distilled water and air dried.

Immunofluorescence staining

Fifteen to 20 μlit of the specimen cell suspension were placed on each slide well. They were allowed to dry at room temperature. The cells were fixed by placing the slides in cold (-20°c) aceton and kept in -20 freezer for 10 min. The excess liquid was drained off and 10 μlit of monoclonal anti RSV IgG conjugated to fluorescein stain (Abbot Company, USA) was added to each well.

The slides were placed in a humidified jar and incubated at 37°c for 1 hr. After the incubation period they were washed in PBS for 3 min, mounted with buffered glycerol (PH 8), and examined with a UV microscope. For positive control, infected RSV cells supplied by the company were similarly stained and for the negative control, similar procedures were used and unimmunized conjugates were applied.

Results

One hundred forty patients of different age groups suffering from respiratory illness suspected of being infected with respiratory syncytial virus were referred to the laboratory of kasra hospital. The patients had respiratory symptoms such as fever, cough, coryza. Some patients had signs of bronchiolitis including wet coughing and bronchial wheezing. A history of sickness from each patient was taken and the information such as seasonal month, age, sex, and duration of the illness were recorded. Some of the specimen which were not taken properly and not suitable for processing were rejected and repeated sampling was requested. Negative control specimen were taken from apparent clinically healthy individuals were also processed and stained. The prepared samples in duplicate wells fixed in aceton were stained using monoclonal antibody against the G protein of RSV surface glycoprotein. The direct IF method was applied and slides containing the specimen and examined by a UV equipped microscope. Positive and negative controls were included in each slides and duplicate

wells containing cells from each specimen were scanned and at least 50 cells were examined for the presence of fluorescence stain. At least 8 cells with fluorescence spots were considered positive. These stain spots appeared as either fine granules or polar cytoplasmic large mass.

From the total of 141 specimen referred for the RSV diagnosis the highest number were during the cold season mainly in January and February and also the majority of positive cases were also from this period (Fig 1).

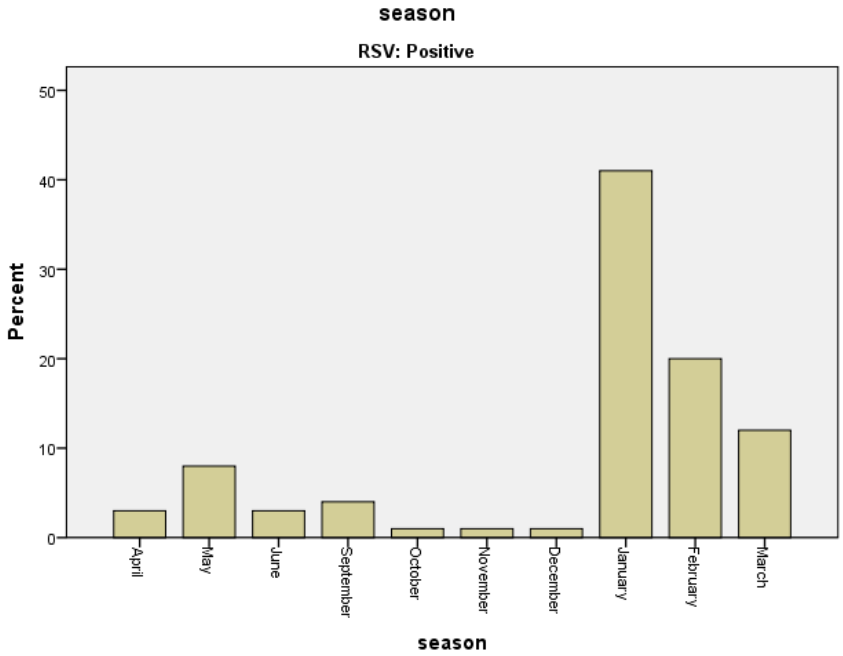

Fig 1. Distribution of positive tests for RSV during the months in 141 admitted children

During this two month almost 60% of the referred patients were positive. During July and Aug there was no infection detected. As it is shown in fig 1 and table 1 the majority of RSV infections were in children within the first 2 month of birth, and only one case was in an adult patient 70 years old which indicated the infection can occur in older people. The was no significant difference between the male and female patients (Table2).

Table 1. Frequency of positive cases based on age of children

Age group(months)	Number	RSV(+)	RSV(+)%
0-2	66	36	58.06
2-8	34	13	20.96
8-16	22	6	9.67
16-32	4	2	3.22
32-36	7	4	6.45
>36	8	1	1.61
Total	141	62	100

Table 2. Frequency of RSV positive cases based on sex

Test	Positive cases	Female (RSV positive %)	Male (RSV positive %)
141	62	52 (57.69)	89 (35.95)

Discussion

Respiratory syncytial virus is a causative agent of severe respiratory disease of infants and young children (4).

It can also affect older people causing respiratory illness in elderly(11) which can be mistaken clinically with infection of respiratory caused by other viruses. The disease in children is usually mild but sometimes it could be severe and life threating which requires hospitalization. The virus is very labile and can be inactivated easily in the environment. Therefore, isolation of the virus using cell culture method is not practical. The lesions produced in infected cells appear as cell fusion and syncytial formation which is calcium dependent phenomenon (17). We used a direct immunofluorescence technique using the specific monoclonal antibody conjugate which detects the presence of viral antigen in infected respiratory cells. Viral antigen in affected cells appears as cytoplasmic granules or as solid polar mass. In previous studies it was reported that RSV infection in newborns and young children was common specially during the cold seasons (15, 16). We found that most of the infections occur during the first 6 month of age. In one case a seventy years old man suffering from flu like disease with nasal and tracheal secretion was positive for RSV. This indicated that the disease could occur in adults with respiratory illness. Perhaps some cases of respiratory infection in adults with common cold clinical manifestation are due to RSV infections.

Although the rate of infection is high during the winter month but this does not mean that during the summer time there is no infection. As it is reported in our study there were 3 case of infection during the month of June. However the results of this study indicated that RSV infection is common and occurs every year during the cold season in young children and new born in Tehran. It is suggested that proper information regarding the rate of occurrence of RSV infection should be brought up to the attention of clinicians and necessary precautions be taken for treatment, control and spread of the disease.

Conflict of Interest

The authors declare that they have no conflict of interest.

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