ORIGINAL ARTICLE

e-ISSN: 2349-0659 p-ISSN: 2350-0964
doi: 10.21276/apjhs.2017.4.4.19

Incidence of respiratory syncytial virus in hospitalized children less than 2 years of age with acute lower respiratory tract infections using multiplex real-time polymerase chain reaction in a tertiary care hospital

Gaganpreet Kaur Grewal[1]*, Bimla Devi[1], Pushpa Devi[1], Karnail Singh[2]

[1]Department of Microbiology, Government Medical College, Amritsar, Punjab, India, [2]Department of Pediatrics, Government Medical College, Amritsar, Punjab, India

ABSTRACT

Background: Respiratory viruses have been found to be the predominant cause of acute lower respiratory tract infections (ALRTIs) in young children, irrespective of climatic zones. In various studies, respiratory syncytial virus (RSV) has been found to be the major pathogen responsible for hospitalizations. The incidence rate of RSV varies according to the latitude, altitude, and climatic conditions of a particular geographical area. **Aim:** The aim of present study was to know the incidence of RSV among hospitalized children with ALRTIs under 2 years of age and identify social and demographic factors associated with severe RSV infection. **Materials and Methods:** Nasopharyngeal secretions were collected using sterile swabs from 50 children. Nucleic acids were extracted using spin column method and detected using real-time PCR. Social and demographic data were collected using preset pro forma. **Results and Conclusion:** Out of total 50 nasopharyngeal samples, 13 (26%) tested positive for RSV. Major predictors for severity of RSV related ALRTI were male gender, birth during winter months and residence in the rural area.

Key words: Respiratory syncytial virus, children less than two years of age, severity of respiratory syncytial virus infection, real-time polymerase chain reaction

INTRODUCTION

Acute lower respiratory tract infections (ALRTIs) are a leading cause of global mortality in young children.[1] According to the WHO report (1993), complicated ALRTIs contribute 30% toward all childhood deaths under 5 years of age. ALRTIs are also the most common cause of hospitalization in young children in developing countries like India. Viruses are more common cause of ALRTIs in young children as compared to bacteria.[2] The most important viruses causing ALRTIs are RSV, Parainfluenza viruses, Influenza A and B viruses, and Adenoviruses. According to the UNICEF/WHO report (2006), RSV is the most common cause of respiratory infections in young children. Novel viruses that have been associated with ALRTIs in children are human metapneumovirus, bocaviruses, and coronaviruses.

Before 1980s, only conventional diagnostic methods were available for viral diagnosis; therefore, the role of viruses as causative agents of ALRTIs was underestimated. However, now with the availability of rapid antigen-based tests and molecular methods like polymerase chain reaction (PCR) in routine diagnostics, we have come to know the true extent to which viruses contribute toward ALRTIs in children. The presence of focal consolidation on chest X-ray and high levels of procalcitonin in serum (values >0.1 μg/L) point toward bacterial rather than viral etiology. However, the definitive methods of diagnosing viral ALRTIs are tissue culture and molecular methods. Since the tissue culture methods are available only at referral labs., so the only tests that can be routinely used for identification of viruses as an etiological agent of ALRTIs are molecular methods like PCR, which is are also available in multiplex format.[3]

The high-risk group in case of RSV comprises children with severe underlying comorbidities such as congenital heart disease (CHD), pulmonary hypoplasia, cystic fibrosis, Down syndrome, neuromuscular disease, and immunocompromised status. The proportion of children hospitalized with RSV from this group is high, but a large number of hospitalizations with severe RSV infection are of previously healthy young infants.

According to the WHO, the most significant social and demographic factors associated with the development of severe ALRTIs in children are malnourishment, low birth weight, non-exclusive breastfeeding during first 6 months of life, overcrowding and indoor air pollution.

In this study, we determined the incidence of RSV in the study population using multiplex real-time PCR along with data in a

Address for correspondence:
Dr. Gaganpreet Kaur Grewal, Department of Microbiology, Government Medical College, Amritsar, Punjab, India. Phone: +91-7837425421.
E-mail: ggrewal651@gmail.com

Received: 10-09-2017 **Revised:** 25-09-2017 **Accepted:** 31-10-2017

bid to identify social and demographic factors most relevant in our geographical area (Punjab, India).

MATERIALS AND METHODS

The study was undertaken in the Department of Microbiology, Government Medical College, Amritsar. The study population comprised children below 2 years of age hospitalized with ALRTIs in Pediatrics Department of Bebe Nanki Mother and Child Care Centre, Amritsar. Samples were collected after written and informed consent from the parents along with history. A total of 50 nasopharyngeal swabs were collected and brought to microbiology lab. for further processing. The socioeconomic status was assessed using Kuppuswamy's socioeconomic scale 2017, malnutrition status was decided using Indian Academy of Pediatrics classification, and severity of ALRTI was accessed by oxygen saturation on presentation and IMNCI/WHO clinical criteria. *P* value was calculated using Chi-square test.

Inclusion and Exclusion Criteria for the study Group

Inclusion criteria

Indoor patients diagnosed with ALRTI below 2 years of age, on examination had sibilant rales on auscultation, chest radiograph was suggestive of bronchiolitis (horizontalization of the dimensions, widening of the intercostal spaces, trapping of air, and flattening of diaphragmatic hemi-cups).

Exclusion criteria

Indoor patients with ALRTIs age >2 years, patients with ALRTIs with duration of illness >5 days or with focal consolidation on chest X-ray on presentation or children hospitalized with recurrent wheezing. All cases of dyspnea with sibilance which could be explained by other causes such as heart defects and intrapulmonary foreign body.

Sample Collection and Transportation

Nasopharyngeal secretions were collected using cotton-tipped swabs, and normal saline was used as transport media. Immediately after collection, the swabs were transported to swine flu lab. In a box lined with ice packs and were stored at −70°C until further processing. The nucleic acids were extracted from samples using spin column method within 24 hours of collection. Presence of RSV was detected using multiplex real-time PCR kit from fast-track diagnostics (FLU/HRSV). The kit could also detect Influenza A and B viruses in the samples.

Extraction of Nucleic Acids

The extraction was performed using RTP pathogen kit based on spin-filter format (a type of physical extraction process).

Real Time PCR

Principle of multiplex real-time PCR kit: The viral RNA extracted from respiratory samples is transcribed into cDNA using specific primer mediated reverse transcription step, followed by in the same tube by PCR. The presence of specific pathogens sequence in the reaction is detected by an increase in fluorescence observed from the relevant dual-labeled probe and is reported as cycle threshold value (ct) by the real-time thermocycler. The assay uses Brome mosaic virus as an extraction or internal control (IC). The IC is added to each sample and the negative control at lysis buffer stage of the extraction process.

Method

After completion of the extraction process according to the kit instructions, the eluted nucleic acids from the sample were immediately put on to the ice. Following the instructions from the manual of the PCR kit, a master mix was prepared by mixing following constituents according to the formula:

Buffer=12.5 µl × (n + 2)

Enzyme=1 µl (n + 2)

Primer probe mix=1.5 µl (n + 2)

Where n=number of samples being processed at a time.

The PCR tubes were labeled, and 15 µl from master mix tube was added to each tube and from the extracted sample 10 µl of elute was added to its respective PCR tube (thawed and vortexed before use). The contents of tubes were mixed by pipetting them up and down. Each tube was vortexed and centrifuged before they were put into the thermocycler (Bio-Rad CFX96). The PCR program was fed into the system beforehand. After loading the thermocycler, lid was closed, the protocol and plate setup were selected from the monitor attached, and the program was given "run" command. Once the "run" was completed, the results were automatically displayed on the monitor with an option for advanced analysis. The positive and negative control traces were analyzed to see if they had met validation criteria. After that, the samples showing exponential fluorescence trace for VIC (green) fluorescent dye were labeled RSV positive.

RESULTS

A total of 50 children (30 males and 20 females) below 2 years of age were tested for RSV using multiplex real-time PCR. Out of 50 samples, 13 (26%) were found to be positive for RSV infection. Out of 13 RSV positive samples 11 (85%) were males and only 2 (15%) were females, 10 (77%) were born during winter months (December to February) and 3 (23%) during non-winter months (March to November), only 2 (22%) were premature and none of them had any underlying congenital heart disease. 10 out of 13 (77%) were from rural area and 3 (23%) from urban area. 9 of them (70%) had at least one older sibling living with them and 4 (30%) had no older sibling. Of 13 positive patients 7 (54%) were severely malnourished, 9 (70%) belonged to lower class, 4 (30%) belonged to low birth weight category, i.e. weighing <2.5 kg at birth. Out of 13 RSV positive patients, 9 (70%) were being breastfed or were exclusive breastfed during first 6 months of life (Graph 1).

DISCUSSION

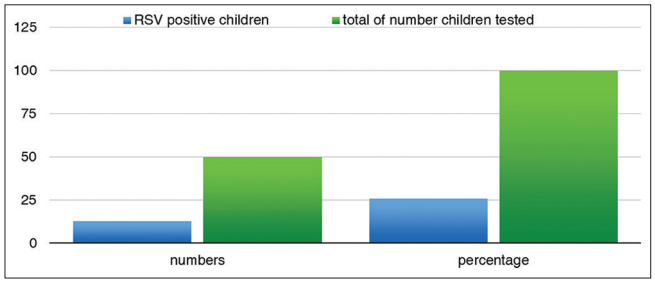
In our study incidence of RSV was found to be 26%, which is in concordance with other studies conducted in India, like in a study by Bharaj *et al*. it was found to be 20.26%[4] and in another study by Singh *et al*. it was 21.3%.[5]

A number of factors were considered in our study to predict the severity of RSV infection, some of these factors showed strong

Table 1: Showing statistically significant risk factors (P<0.05) associated with severe RSV infection

Variables	RSV positive patients (13) (%)	RSV negative patients (37) (%)	*P*
Place of residence			
Rural residence	10 (77)	14 (38)	0.0152
Urban residence	3 (23)	23 (62)	
Gender			
Male children	11 (85)	19 (51)	0.0238
Female children	2 (15)	18 (49)	
Birth season			
Birth during winter season	10 (77)	16 (43)	0.0365
Birth during non-winter season	3 (23)	21 (57)	

RSV: Respiratory syncytial virus



Graph 1: Showing incidence of RSV

correlation with severe RSV infection requiring hospitalization. In Table 1 only statistically significant risk factors (P < 0.05) are reported. In our study, male preponderance (85%) was seen in RSV positive group (P =0.0238), which is in concordance with other studies, such as García *et al.*[6] and Singh *et al.*[5] The probable explanation for this may be that boys have anatomically narrower and shorter airways, and physiologically too their lungs lag behind in development compared to those in females, which makes them susceptible to all ALRTIs in general (irrespective of etiological agent).[7]

In our study10/13 (77%) were born during the winter season (December–February). Birth during winter months was found to strong predictor for RSV related disease severity (P =0.0365). As RSV outbreaks occur during winter months in temperate areas[8] (winter of subtropical areas is similar to those in temperate areas), these children are placed at higher risk of developing severe RSV infection because they have high probability of being infected at a relatively younger age when immune system is immature, and lungs are still developing. As the protective antibodies against RSV are transferred to the fetus during the third trimester and are usually short-lived these children are less likely to acquire circulating antibodies from their mothers through placenta as compared to children born after the winter season.

Third factor which showed high concordance in RSV positive children was rural residence (P =0.0152). 10 out of 13 (77%) RSV positive children in our study belonged to rural areas. In rural areas majority of cooking is still carried by burning wood and cow dung cakes as a fuel compared to cities where natural gas or kerosene are commonly used. It is known fact, that burnt wood emanates toxic fumes containing polycyclic aromatic hydrocarbons, acrolein, benzene, formaldehyde, etc. These toxic substances when inhaled cause cytotoxic damage to the lungs and make these children highly susceptible to ALRTIs especially due to RSV.[9]

In our study, we did not find a protective effect of breastfeeding against RSV related severe ALRTIs. This may be due to small sample size of our study. Other studies have shown a protective effect of breast milk against RSV related ALRTIs in infants (Baker *et al.*[10] and Oddy *et al.*)[11] However, a study by Erin Tarter 2002 which failed to show a protective effect of breastfeeding.[12] Only 2 children in our study group were born premature, and none of the children included in the study had underlying CHD, pulmonary dysplasia, or immunocompromised status.

Our study has an inherent socioeconomic bias because most of the children included in our study group were from lower socioeconomic status and only a few belonged to middle or upper socioeconomic status. This is so because only people from lower socioeconomic status are likely to seek medical services at a government institute like ours. Therefore, socioeconomic status could not be analyzed as a risk factor.

CONCLUSION

In our study incidence of RSV was found to be 26% (13/50); thus RSV is a major contributor toward hospitalization related to ALRTIs. The major predictors of RSV related severe LRTIs were male gender, birth during winter season and residence in a rural area. It is imperative to understand high-risk groups to focus specific preventive strategies, like possible new vaccines at them.

REFERENCES

1. Tregoning JS, Schwarze J. Respiratory viral infections in infants: Causes, clinical symptoms, virology, and immunology. Clin Microbiol Rev 2010;23:74-98.
2. Broor S, Parveen S, Bharaj P, Prasad VS, Srinavasulu KN, Sumanth KM, *et al*. A prospective three-year cohort study of epidemiology and virology of acute respiratory infections of children in rural India. PLoS One 2007;2:491-3.
3. Mahony JB. Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 2008;21:716-47.
4. Bharaj P, Sullendra WM, Kabra SK, Mani K, Cherian J, Tyagi V, *et al*. Respiratory viral in factions detected by multiplex pcr among patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. Virol J 2009;5:901-16.
5. Singh AK, Jain A, Jain B, Singh KP, Dangi T, Mohan M, *et al*. Viral etiology of acute lower respiratory tract illness in hospitalized pediatric patients of a tertiary hospital: One year prospective study. Indian J Med Microbiol 2014;32:13-8.
6. Garc4;ioll Bhore R, Soriano-Fallas A, Trost M, Chason R, Ramilo O, *et al*. Risk factors in children hospitalized with

rsv bronchiolitis versus non-rsv bronchiolitis. Pediatrics 2010;126:1055-7.
7. Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988;319:1112-7.
8. Schanzer DL, Langley JM, Tam TW. Hospitalization attributable to influenza and other viral respiratory illnesses in Canadian children. Pediatr Infect Dis J 2006;25:795-800.
9. Morris K, Morgenlander M, Coulehan JL, Gahagen S, Arena VC. Wood-burning stoves and lower respiratory tract infection in American Indian children. Am J Dis Child 1990;144:105-8.
10. Baker D, Taylor H, Henderson H. Inequality in infant mortality: Causes and consequences in England in the 1990s. J Epidemiol Community Health 1990;52:451-8.
11. Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, *et al*. Association between breast feeding and asthma in 6 year old children: Findings of a prospective birth cohort study. Br Med J 1999;319:815-9.
12. Tarter EE. Factors Affecting Respiratory Syncytial Viruspositive Wheezing Illness in Infants [Thesis for Master of Science Degree].The Graduate School: University of Wisconsin Stout; 2002.

How to cite this Article: Grewal GK, Devi B, Devi P, Singh K. Incidence of respiratory syncytial virus in hospitalized children less than 2 years of age with acute lower respiratory tract infections using multiplex real-time PCR in a tertiary care hospital. Asian Pac. J. Health Sci., 2017; 4(4):77-80.

Source of Support: Nil, **Conflict of Interest:** None declared.