

Respiratory Syncytial Virus in Lower Respiratory Tract Infections

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Abstract

Objective: Acute lower respiratory infections lead to high morbidity and mortality rates in children from developing countries. The aim of this study was to look into the extent of *respiratory syncytial virus* infections in children with special reference to the role of specific immunoglobulins in protection against infection as well as the association with bacterial pathogens.

Material & Methods: Nasopharyngeal aspirates were tested for *respiratory syncytial virus* antigen by enzyme immunoassay and IgA antibodies by single radio immunodiffusion test. Viral culture on HEP-2 cell system and bacterial culture was done. Sera were tested for detection of antibodies to *respiratory syncytial virus* by indirect fluorescent antibody test. Antigens of *streptococcus pneumoniae* and *haemophilus influenzae* were detected in serum and urine by latex agglutination assay.

Findings: Incidence rates of acute lower respiratory infections were highest in infants; bronchiolitis and bronchopneumonia being the main contributors. *Respiratory syncytial virus* infection was found in 27.08% of the cases.

Conclusion: Secretory IgA antibodies level was found to be a good indicator of *respiratory syncytial virus* infection as seen by the significantly higher levels in cases as compared to both non respiratory syncytial virus cases and controls.

Key Words: Secretory IgA, Respiratory syncytial virus, Respiratory infections, Bronchiolitis, RSV

Introduction

Acute lower respiratory infections (ALRI) are responsible for high rate of morbidity and mortality in children in developing countries^[1].

The impact, however, has been largely ignored as compared to the other two major causes of child mortality -- diarrhoea and malnutrition. This disparity has been partly attributed to the many pathogens causing ALRI with overlapping clinical syndromes and the inability of

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even the most advanced research laboratories to detect pathogens in more than half of the cases.

High incidence of this problem has been recorded in both urban and rural children in India, as elsewhere. *Streptococcus pneumoniae* (*S.pneumoniae*) and *Haemophilus influenzae* (*H.influenzae*) are commonly encountered bacterial pathogens and *respiratory syncytial virus* (RSV), the predominant viral pathogen^[2]. RSV is currently the leading viral pathogen associated with bronchiolitis and lower respiratory tract disease requiring hospitalization in infants and young children worldwide^[3]. Rapid identification is desirable for the interruption of the chain of infection and for the prompt application of specific antiviral therapy^[4]. The extent of RSV infections in children with special reference to the role of specific immunoglobulins in protection against infection as well as the association with bacterial pathogens need to be looked into. It is also essential to analyze the new nonconventional rapid techniques for diagnosis. Hence, the present study was undertaken.

Material & Methods

The study was conducted on 50 children below the age of 2 years, suffering from ALRI admitted to the pediatric ward of Lok Nayak Hospital, New Delhi. Twenty-five healthy children were selected as controls from those attending well baby and immunization clinics. Children were defined as having ALRI if at least one of the following signs could be detected: rales/crepitations, wheezing, stridor, fast breathing, cyanosis or chest indrawing.

Besides the routine investigations, a detailed history was recorded on a preset performa and a physical examination was carried out on each one of them. Assessment of nutritional status was done according to the criterion laid down by the Indian Academy of Pediatrics Nutritional sub-committee (1972).

Nasopharyngeal aspirates (NPA), collected using standard method^[5], were rinsed into the mucous trap with 3 ml of Hank's balanced salt

solution supplemented with antibiotics and transported to the laboratory on ice. The contents were vortexed and 300 μ l of this suspension was aliquoted and stored at -70⁰C for RSV antigen detection by solid phase enzyme immunoassay (RSV-EIA) manufactured by Abbott Laboratories. The remaining NPA was centrifuged at 1500 rpm for 10 minutes and 0.2 ml of the resulting supernatant was stored at -20⁰C for estimation of IgA antibodies using single radioimmunoassay test. The rest of the supernatant was used for viral culture on the HEP-2 cell system and the tube cultures were screened daily for cytopathogenic effects (CPE) for a maximum of 28 days. If CPE was observed in the cell culture tubes, the haemadsorption test was performed.

A part of the NPA was directly transferred to a sterile container and transported to the laboratory at room temperature for bacterial culture. NPA was cultured on 5% sheep blood agar, chocolate agar and MacConkey's agar and incubated aerobically in 5-10% CO₂. Organisms were identified on the basis of colony characteristics, gram staining and biochemical reactions. Further confirmatory tests were carried out as prescribed by Mackie and McCartney^[5].

Blood was collected (3-5 ml) aseptically by venipuncture on the day of admission and after 2-3 weeks, serum was separated and stored at 20-25⁰C until tested for the detection of antibodies to RSV by the indirect fluorescent antibody (IFA) test using VirgoTM reagents manufactured by Electro Nucleonics and antigens of *H. influenzae* and *S. pneumoniae* by latex particle agglutination assay using bioMerieux Lab reagents, France.

Urine collected in sterile container, after being mixed with cold 95% ethanol (3:1) and kept at 5⁰C for 1 hour, was centrifuged at 2500 rpm for 20 minutes. The supernatant was discarded, tube dried for 30 minutes, normal saline added to the pellet and centrifuged again. Supernatant was tested for antigen of *H. influenzae* and *S. pneumoniae* by latex particle agglutination assay using bioMerieux Lab reagents, France.

Findings

Out of the 50 children 2 cases have been excluded from analysis due to reasons discussed later. It was noted that 87% of the children were less than 1 year of age; more than 60% were less than 6 months old. The majority of children were males (77%). ALRI occurred throughout the year, but RSV infection occurred essentially in the winter season.

As regards the grouping of the ALRI cases on the basis of clinical diagnosis, the dominant cause of infection was bronchopneumonia (54.8%) followed by bronchiolitis (40.5%), among the children of age less than one year. The dominance of bronchopneumonia persisted in the children aged more than 1 year (66.7%), whereas that of bronchiolitis decreased appreciably (16.7%). The occurrence of cases of bronchial asthma and croup was far less in both the age groups (2.4% for both in less than 1 year age group and 0% for croup and 16.7% for asthma in more than 1 year age group).

The various organisms isolated from nasopharyngeal aspirates of ALRI cases and controls are listed in Table 1. A comparison of the cases and controls clearly indicates that the colonization of the respiratory tract by potential pathogens was significantly higher in children afflicted with ALRI.

The infection rates, regardless of age, for males and females do not show any significant difference as revealed by the chi square test. Furthermore, in no age group were either males or females preferentially affected. An identical trend was seen for all the clinical features studied.

Of the 50 cases studied for the presence of RSV antigen by ELISA, 13 were positive i.e. 27%. All the controls tested negative. Two had optical density in the gray zone (between $\pm 7\%$ of cut off value) and they could not be retested and were excluded from the statistical analysis. As to the age and gender related incidence of RSV as detected by ELISA, there was a markedly high incidence of RSV infection in children aged 6-9 months with a concentration of cases in children below 9 months of age.

Table 1: Organisms isolated from NPA^[a] of children with ALRI^[b] and controls

Sr. No.	Organism isolated	No. Of pts. (%) (Total 48)	No.of controls (%) (Total 25)
1.	Streptococcus pneumoniae	7 (14.6)	1 (4)
2.	Haemophilus influenzae	4 (8.3)	0
3.	Staphylococcus aureus	5 (10.4)	0
4.	Pseudomonas aeruginosa	5 (10.4)	2 (8)
5.	Escherischia coli	20 (41.7)	3 (12)
6.	Klebsiella species	7 (14.6)	1 (4)
7.	Other Non Lactose Fermenting organisms	7 (14.6)	1 (4)
8.	Candida species	14 (29.2)	4 (16)
9.	Serratia marcessens	1 (2.1)	0
10.	Streptococcus viridans and Niesseria catarrhalis	5 (10.4)	8 (32)

[a] NPA- Nasopharyngeal aspirate

[b] ALRI- Acute lower respiratory tract infection

Table 2: Results of LPA^[a] assay for detection of antigen of *S. pneumoniae* and *H. influenzae* in serum and urine samples of ALRI^[b] cases

Organism	Sample	Age in months				Total
		0-6	6-12	12-18	18-24	
Streptococcus pneumoniae	Serum	3	3	0	1	7
	Urine	2	3	0	1	6
Haemophilus influenzae	Serum	6	0	0	1	7
	Urine	4	0	0	1	5

[a] LPA- Latex particle agglutination

[b] ALRI- Acute lower respiratory tract infection

Also 21.6% males and 45.5% females were found to have RSV infection, though no significant difference was seen in these values.

A comparison of the results of RSV antigen detection by ELISA (in NPA) and RSV-specific IgG detection by IFA (in serum) is shown in Table 3. It was seen that 34 out of 48 (70.8%) children with ALRI and 18 out of 25 controls (72%) had serum RSV-specific IgG antibodies. Of the 34 cases and 48 controls with detectable antibodies at a titre of 1:8, 9 cases and 1 control had detectable antibodies at a titre of 1:256. Of these 10 children 7 showed a fourfold rise in titre of IgG antibody.

The secretory IgA values of RSV cases were higher than that of other cases of ALRI and controls. The children with RSV in the age group 0-6 months had a significantly lower

value of secretory IgA than children aged 6-24 months.

Serum and urine were tested for the presence of antigens of *S. pneumoniae* and *H. influenzae* by latex particle agglutination assay (LPA). The results are shown in table 2. Out of the 7 serum samples that tested positive for antigen of *S. pneumoniae* by LPA, in 4 cases the organism was isolated from the NPA and 6 had detectable antigen in the urine also. Three cases were negative for LPA though the organism was isolated from NPA. Whereas out of the 7 serum samples positive for *H. influenzae* antigen, urine of 5 was positive for antigen and in 3 cases the organism was isolated from the NPA. In 1 case the NPA yielded both *S. pneumoniae* and *H. influenzae* antigens but it was not detected by LPA in

Table 3: A comparison of the results of RSV^[a] antigen detection by ELISA^[b] (inNPA^[c]) and RSV-specific IgG detection by IFA^[d] (in serum)

Organism	Age groups (months)				Total
	0-6	6-12	12-18	18-24	
Antigen +ve and four fold rise of antibody present	4	1	0	2	7
Antigen +ve and four fold rise of antibody present	2	4	0	0	6

[a] RSV- Respiratory syncytial virus

[b] ELISA- Enzyme linked immunosorbent assay

[c] NPA- Nasopharyngeal aspirate

[d] IFA- Indirect fluorescent antibody test

either serum or urine. None of the controls showed the presence of any antigen.

Twenty random samples of NPA were cultured on HEp-2 cell line. This was not done for all cases since the cell line could not be maintained during the whole duration of the study. In 2 of these, syncytium formation was seen, 1 on the fourth and another on the fifth day. These were found to be caused by RSV due to the absence of haemadsorption by guinea pig red blood cells to the cell culture system. These 2 specimens were also positive by ELISA for antigen detection. One specimen which was positive for antigen by ELISA was not detected by cell culture.

Out of 48 cases enrolled, 45 recovered and 3 died (2 had congenital heart disease with bronchopneumonia and 1 had bronchiolitis with low weight) resulting in the case fatality rate of 6.25%.

Discussion

In our study, the concentration of ALRI cases in the first year of life (87%) is similar to the pattern seen elsewhere in India^[6] and in other developing countries^[2]. The preponderance of male babies suffering from ALRI (77%) in a hospital-based study is probably not a true indicator of the situation in the community. A similar occurrence has been seen in other developing countries and has been attributed to the tendency of parents to bring sick male babies to the hospital earlier than female babies. As in our study, Joshi and Coworkers from India have also reported the rise of ALRI cases in winter^[7].

A high percentage of these cases had antigen of *S. pneumoniae* and *H. influenzae* in body fluids (29.2%). Numerous authors in India and other developing countries have also laid stress on the important role of these two pathogens^[6,7]. Pharyngeal colonization by these organisms was not necessarily found to be associated with the presence of antigen of any invasive strain in either serum or urine. A

similar observation has been made by Hortal and Coworkers^[8].

RSV infection was seen in 27.08% of cases of ALRI. Other studies from India have reported incidences from 6-36% in cases of ALRI^[3,6]. It was seen that a very high percentage of cases of RSV infection occurred in children below one year of age. Earlier studies in India and abroad have clearly pointed to this age group as a target for RSV infection^[6,9].

In our study, 27.08% cases tested positive by ELISA. It appears that with the exception of our study^[10], which used virus isolation, most studies using various techniques other than ELISA have reported a low incidence of RSV infection. Virus isolation detected 2 cases of RSV but missed one, which had antigen of RSV as detected by ELISA. This specimen probably gave negative results because it was frozen during transportation, thus rendering the virus nonviable but still detectable by ELISA. This points to the superiority of this assay as has also been observed by Masters and Coworkers^[11].

A significantly high level of secretory IgA was estimated from NPA of RSV cases and compared to children with other causes of ALRI and controls. McIntosh and Coworkers^[1] have reported values ranging from 2-50 mg/100ml, which is similar to ours. A protective role has been attributed to the secretory IgA and its tremendous rise in cases with RSV indicates that secretory IgA levels are a good indicator of RSV infection.

Conclusion

RSV is an important etiological agent causing bronchiolitis and bronchopneumonia and the exposure is virtually universal during childhood with a very high percentage of cases occurring in children below one year of age. Though more studies are required to evaluate the various diagnostic modalities available, our study concludes that a combination of various rapid and reliable techniques used here may provide important diagnostic information in establishing the etiology.

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