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Original Research Article

Detection of respiratory syncytial virus in cases of acute lower respiratory tract infection in pediatric age group by antigen detection and PCR analysis in a tertiary care hospital of eastern Odisha

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ABSTRACT

Background: Respiratory Syncytial Virus (RSV) is one of the most common causes of acute lower respiratory infection (ALRI) in the paediatric age group. Various demographic and risk factors are associated with the severity of the disease.
Aim & Objective: This study was conducted to detect RSV from ALRI cases using two different methods and to correlate the findings with various risk factors.
Materials & Methods: The study was carried out over a period of 2 years on 242 ALRI cases in the Paediatrics Department of SCB Medical College and Hospital, Cuttack. Nasopharyngeal aspirates were collected using standard procedures and subjected to a rapid immunochromatographic test (ICT) for antigen detection, and molecular detection was performed using PCR.
Results: Clinical and demographic data were collected and analyzed. Among the 242 clinically diagnosed ALRI cases, 14.05% were positive for RSV by one or both methods (ICT and RT-PCR). Considering real-time RT-PCR as the gold standard, the sensitivity, specificity, PPV, and NPV of the ICT were found to be 82.35%, 100%, 100%, and 97.19%, respectively. There was a significant association between risk factors such as prematurity, vitamin A supplement deficiency, and smokers in the household with RSV infections (p-values of 0.001, 0.003, and 0.002, respectively).
Conclusion: The study demonstrates two different methods for identifying RSV; thus, a simple, rapid method can be used as an alternative in resource-limited settings, enabling clinicians to avoid unnecessary antibiotic prescriptions in such cases. It also highlights risk factors that are adversely associated with disease morbidity, whose efficient control can significantly reduce the disease burden.

1. Introduction

Acute lower respiratory infections (ALRIs) are a leading cause of morbidity, mortality and hospital admission in paediatric age group.[1] World Health Organization (WHO) estimates that nearly 4 million deaths per year, occurs due to ALRI.[2] About 90% of ALRIs occur in developing countries and are a leading cause of mortality especially in children under 5 years age group.[3,4] Among children hospitalized with ALRI in India, half the infections are of viral etiology.[5–7] So, there is a need to focus on this grave situation.

Although bacterial pathogens play a major role as primary or secondary cause of severe lower respiratory tract disease in developing countries, respiratory viruses are prevalent and probably primary causal agents of most

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acute respiratory infections.[8,9] Respiratory viral infections are studied extensively in developed countries and their impact on health care is well understood. There is a huge gap of information on the burden of respiratory viral infections in developing countries.[10] Among the viral etiological agents respiratory syncytial virus (RSV) is a well-recognized and most important pathogen causing acute respiratory disease in infants and children and associated with significant disease burden in terms of hospitalization, related complications and mortality.[11,12]

RSV, classified in Pneumovirus genus of Paramyxoviridae family, is an enveloped virus with a negative sense single stranded RNA genome which encodes for 11 proteins.[13] Two major antigenic groups RSV-A and RSV-B, have been described on the basis of monoclonal antibodies against G and F glycoproteins and molecular differences of several genes.[14–16] RSV causes repeated infections throughout life due to limited immune protection from earlier RSV exposure and genetic variability.[16–18]

In India, RSV has been identified as an important cause of lower respiratory tract infections (17 to 32%) in paediatric age group.[6,7] Hence the current study aims to identify RSV by two different methods in clinically diagnosed cases of ALRI in under 5 age group.

2. Materials and Methods

2.1. Study design

A prospective study was conducted in the department of Microbiology, S.C.B. Medical College & Hospital, Cuttack in collaboration with SVPPGIP, SCB Medical College & Hospital, Cuttack and RMRC, Bhubaneswar over a period of 2 years between June 2016 to June 2018. Ethical clearance was obtained from the institutional ethical committee and consent was taken from the parents. The study group comprised of 242 clinically diagnosed cases of ALRI either presenting to outpatient department (OPD) or admitted in paediatric ward.

2.2. Inclusion criteria

Children less than 5 years were included using standardized ALRI case definition of WHO.[19,20]

2.3. Exclusion criteria

Children known to be affected by congenital heart disease, tracheosophageal fistula, anatomical defects and genetic abnormalities and whose parents refused to give consent were excluded in this study.

2.4. Data collection

Children ≤ 5 years presenting with symptoms of ALRI in the OPD or admitted in the paediatric ward and PICU were studied. Data such as age, gender, date of visit to OPD or admission to the ward, duration of symptoms and relevant detailed history was collected with regards to family history of ARI & smoking, antenatal history, vitamin A supplement. Detailed general physical examination, followed by examination of respiratory system was performed and the findings were recorded. Relevant blood investigations and chest-x ray findings were noted. Statistical analysis done by SPSS version 26 (IBM Corporation, New York, USA) software.

2.5. Sample collection & transport

Nasopharyngeal aspirates were collected aseptically in sterile aliquots. The aspirate was added with 2.5 ml normal saline and was transported in vaccine carrier with ice packs, to maintain cold chain.

2.6. Laboratory test

Immunochromatographic test was done using SD BIOLINE (Accor. To Manufacturer's instruction) one step RSV antigen test and the result was interpreted for presence of RSV antigens by appearance of test band and control band over the test strip.

2.7. PCR analysis

The remaining sample was then added to Hi-media Viral transport medium and stored at -20ºC till it was transferred to Viral Research and Diagnostic Laboratory (VRDL) of RMRC, Bhubaneswar for PCR analysis.

2.8. Extraction of the RNA

QIAMP viral RNA isolation kit from Qiagen (QIAGEN, Germany) was used for extraction of RNA as per manufacturer's instructions.

2.9. Real time reverse-transcriptase (RT) PCR

Details of primers and probes used for respiratory syncytial virus identification by real time RT-PCR detection was carried out in ABI 7500 Real -Time PCR Instrument (Applied Bio systems). (Table 1)

The samples were considered positive if reaction growth curve cross the threshold with a sigmoid shape curve and CT value of 29 indicating strong positive reaction due to presence of abundant nucleic acid of the virus.

3. Results

Out of 242 clinically diagnosed ALRI cases, 34 (14.05%) were positive for RSV by one or two of the different methods (Antigen detection by ICT or/ & real time RT PCR). Out of which majority of RSV positive cases were in the age group of less than 6 months i.e. 14 (41.18%). Male preponderance was seen among the RSV positive cases

i.e 19 (55.89%) while 15 cases (44.11%) were females, with Male: Female ratio was found to be1.27 (Figure 1). Majority of RSV positive cases were from paediatric ward 16 (47.06%) followed by outpatient department 14 (41.18%) followed by paediatric ICU 4 numbers (11.76%). Seasonal variation was seen in the study as a greater numbers of RSV positive cases were seen in August i.e. 9 (26.47%) and July i.e. 7 (20.58%), followed by September where it was 5 (14.71%).

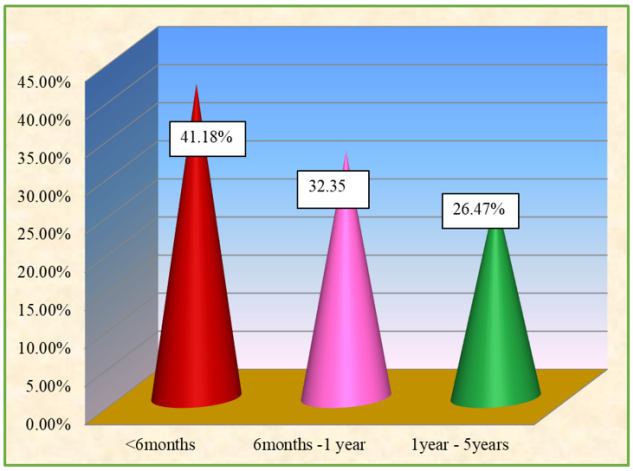

Figure 1: Age distribution of RSV positive cases (n=34)

Bronchiolitis was the major clinical diagnosis among RSV positive cases i.e in 20 (58.82%), followed by Pneumonia in 8 (23.52%) and Asthma in 6 (17.64%) cases.

Cough, Rhinorrhoea and fever were the common symptoms associated with RSV infection which were seen in 33 (97.05%), 28 (77.78%) & 24 (70.06%) of cases respectively in this study. Chest indrawing 28 (82.35%) and nasal flaring 14 (47.06%) were the major clinical signs found among RSV positive cases (Table 2). Out of 242 ALRI cases, 28 (11.57%) were found positive for RSV antigen by immunochromatographic test and 214 (88.43%) were negative. However, RSV was detected in 34 (14.05%) cases from the total cases of ALRI by real time RT PCR.

On comparing ICT with Real time RT PCR, ICT was found to be positive in 28 (11.57%) cases while Real time RT PCR was positive in 34 (14.05%) cases. In 6 (2.48%) cases, though ICT was negative, PCR was found to be positive. p value was calculated by McNemar chi square test and was found to be 0.031 which was statistically significant (Table 3). Considering real time RT PCR as the gold standard method sensitivity, specificity, positive predictive value and negative predictive value of the immunochromatographic test was found to be 82.35%, 100%, 100% and 97.19% respectively.

Among the different risk factors, family history of ARI was seen in 14 cases (41.17%), Prematurity in 11 cases (32.35%), Vitamin A supplement not received by children was seen in 10 cases (29.41%) which contributed to the severity of the disease. p value of 0.001 was observed in prematurity while it was 0.003 for children not received vitamin A supplement which signifies that these patients are more likely to develop acute lower respiratory infections by RSV. There was also significant association of smokers in house with causation of RSV infections in our study with p value of 0.002. (Table 4)

Among the RSV infected patient 32 cases (94.12%) survived while 2 cases (5.88%) died.

4. Discussion

In this study RSV virus was detected in 34 (14.05%) cases out of 242 ALRI cases, which is similar to the study by Yadav et al but varies from various other studies conducted in different parts of world.[2,21,22]The divergence in RSV epidemiology in different parts of the world may be related to variations in climatic conditions, environmental factors and severity of its epidemics from one year to another. In this study, the virus was detected more in the age group of less than 6months i.e 14 cases (41.17%). This is in concordance with the previous studies done indicating the younger age affliction, in RSV infections.[23] The ratio of male to female in RSV positive cases was found to be 1.3:1 which is concomitant with the previous studies.[22,24] This pattern indicates males were probably at more risk of developing RSV infection than females.

In this study, the proportion of RSV positive cases attending paediatric outpatient department were 41.18%, while the inpatients RSV positive cases comprised of 47.06% from paediatric ward and 11.76% from paediatric ICU. While other studies found 23.6% of RSV positive cases from paediatric OPD and 40.2% from hospitalized cases.[13] These variations may be due to difference in the clinical presentation and severity of the illness at the time of seeking medical advice.

There was seasonal variation in the epidemiology of the virus with maximum cases occurring in rainy season (July to September) followed by fall period (January). In the year 2017, maximum RSV positive cases were seen in the month of July and August comprising of 9 (20.58%) & 7 (26.47%) cases respectively, followed by January where it was 4 (11.76%). While in 2016 maximum cases were found in August and September consisting of 4(11.76%) & 5(14.71%) cases respectively. However, the study of Yadav et al (2016) reported high prevalence (13.97 percent) of RSV infection from Nov-Feb with a peak during month of December.[21] Zhang et al(2010) reported majority of the cases (79%) during winter and spring seasons (November to March), while Tariq et al (2016) found highest frequency of RSV infections in January (21 cases [25.9%]). These seasonal variations in RSV infection may be related to a region's climate and demographic factors.[22,25]

Table 1: Details of the primers & probes for RSV detection by real time RT PCR

RSV Virus	Sequence of Primers and Probes	Target Genes	Final concentration of Primers and Probes(nm)
F1	TGGAAACATACGTGAACAARCTTCA	Nucleoprotein	500
R1	GCACCCATATTGTWAGTGATGCA	Nucleoprotein	500
Probe 1	JOEN / CGAAGGCTCCACATACACAGCWGCTGT / 3BHQ_1/	Nucleoprotein	150

Table 2: Symptoms and signs associated with RSV positive cases (n=34)

Symptoms	RSV positive cases	Percentage (%)
Fever	24	70.06
Sore throat	8	23.53
Cough	33	97.05
Rhinorrhoea	28	77.78
Breathlessness	18	52.94
Signs		
Grunting	2	5.88%
Nasal flaring	14	47.06%
Chest indrawing	28	82.35%
Stridor	2	5.89%
Cyanosis	1	2.94%

Table 3: Comparison of ICT with Real time RT PCR (n=242)

	Real time RT PCR positive	Real time RT PCR negative	Total	p- value
ICT positive	28 (11.57%)	0 (0.0%)	28 (11.57%)	
ICT negative	6 (2.48%)	208 (85.95%)	214 (88.43%)	0.031
Total	34 (14.05%)	208 (85.95%)	242 (100%)	

Table 4: Association of different risk factors observed in ALRI cases

Risk factors	Observed in RSV positive cases (n=34)	Percentage (%)	Observed in RSV negative cases (n=208)	Percentage (%)	p value
ARI in family	14	41.17	76	36.54	0.603
Prematurity	11	32.35	24	11.54	0.001(sig)
Nutritional status (low weight per age)	7	20.59	21	10.09	0.076
Vitamin A supplement not received	10	29.41	117	56.25	0.003(sig)
Smokers in house	8	23.53	15	7.21	0.002(sig)

Bronchiolitis was the major clinical presentation found in this study among total RSV positive cases which consisted of 20 (58.82%) cases. This is in concordance with the earlier studies.[25]

In this study cough, rhinorrhoea and fever were the common symptoms associated with RSV infection which were seen in 97.05%, 77.78% & 70.06% of cases respectively which is in concordance with the previous studies.[21,24] The major clinical signs found among RSV positive cases were chest indrawing and nasal flaring in 82.35% and 47.06% respectively. This is similar to the studies done by Yadav et al (2016) and Nifkar et al (2013);they have reported chest indrawing as the most common sign which was seen in 100% of RSV positive cases.[21,24]

In this study ICT and Real time RT PCR were used for detection of RSV antigen and virus. While ICT was found to be positive in 28(11.57%) cases, real time RT PCR was positive in 34(14.05%) cases. In 2.48% cases, though ICT was negative, PCR was found to be positive. p value was found to be 0.031 which was statistically significant. The results were almost in close approximation to the results of Gupta et al (2012) who reported 8.8% cases of ALRI positive for RSV by ICT and 14.7% by RT- PCR,[26] however the study of Tariq et al (2016) reported 24.3% positive by ICT and 27% positive by RT- PCR, which was higher than

our result.[25] These discrepancy in results may be due to use of different immunochromatography test kits with varying sensitivity. Taking RT-PCR as gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value of the immuno-chromatographic method was found to be 82.35%, 100%, 100% and 97.19% where the sensitivity and negative predictive value of ICT was found to be higher than the previous study conducted.[26]

The present study showed association of different risk factors with RSV positive cases like family history of ARI, prematurity, vitamin A supplement deprivation, nutritional status and smokers in house. Family history of ARI was the most common risk factor, seen in 14(41.17%) cases. However, it is not significant as it was also observed in RSV negative cases. The other risk factors like prematurity, Vitamin A supplement found in 11(32.35%) & 10(29.41%) RSV positive cases respectively had strong association with RSV infections in young infants. In premature babies the explanation is obvious as the lungs are less developed & show reduce immune response against RSV infection. As the majority of the RSV positive cases were in the age group of < 6 months indicating that they have not received the vitamin A supplements which is given at 9 months age, thereby making them more susceptible to RSV infection. Among the RSV positive cases, 8 (23.53%) cases had history of smokers in the house which was significantly higher than those seen in RSV negative cases i.e., 15(7. 21%) (p value of 0.002). This might be due to the fact that tobacco smoke being an environmental pollutant can reduce the local defence mechanism of children thus making them prone for RSV infection. Low nutritional status was observed in 20.59% RSV positive cases but it was not significant as it was also seen in 10.09% of RSV negative cases.

Out of 34 RSV positive ALRI cases in our study, 32 (94.12%) survived while 2 (5.88%) died. Data from a hospital-based African study by Eftyhia et al have found a relatively low mortality rate of 2% due to RSV with RSV incidence of 28%.[27]

Finally, our study emphasized on the use of a simple and rapid method for screening and early detection of the virus.

5. Limitation of the Study

Even though the study was done a few years back, the comparative analysis of performance of ICT and RT-PCR is relevant even today. Similar studies with newer point of care tests for RSV will be useful.

6. Conclusions

In view of the results obtained by our study it was observed that Respiratory syncytial virus (RSV) is one of the most important pathogens causing severe lower respiratory tract infections in children often requiring hospitalization.

Although the real-time RT PCR allows the quantification of viral nucleic acids from the clinical sample and is more sensitive, but its high cost, technical expertise and non-availability at resource poor laboratories limits its use. So, an easy and rapid test like immunochromatography can be used as an alternative method for diagnosis of the virus as a point of care test. Furthermore, there was significant association of different precipitating risk factors like family history of ARI, prematurity, Vitamin A deprivation with the severity of the disease, whose effective surveillance and monitoring can reduce the mortality rate in children <1yr.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Tregoning JS, Schwarze J. Respiratory viral infections in infants causes, clinical symptoms, virology and immunology. *Clin Microbiol Rev*. 2010;23(1):74–98.
2. Singh AK, Jain A, Jain B, Jain B, Singh KP, Dangi T, et al. Viral aetiology of acute lower respiratory tract illness in hospitalised paediatric patients of a tertiary hospital: one-year prospective study. *Indian J Med Microbiol*. 2014;32(1):13–8.
3. Sikolia DN, Mwololo K, Cherup H, Hussein AE, Juma M, Kurui JC, et al. The prevalence of acute respiratory infections and associated risk factors. A study of children under five years of age in Kibera lindi village, Nairobi, Kenya. *J Nal Inst Public Health*. 2002;51(1):67–72.
4. Kabra SK. Acute respiratory tract infection (ARTI) control program. In: Ghai OP, Gupta P, Paul VK, editors. Ghai essential paediatrics. New Delhi: Interprint; 2000. p. 349–50.
5. Jain A, Pande A, Misra PK, Mathur A, Chaturvedi UC. An Indian hospital study of viral causes of acute respiratory infection in children. *J Med Microbiol*. 1991;35(4):219–23.
6. John TJ, Cherian T, Steinhoff MC, Simoes EA, John M. Etiology of acute respiratory infections in children in tropical southern India. *Rev Infect Dis*. 1991;13(Suppl 6):S463–9.
7. Maitreyi RS, Broor S, Kabra SK, Ghosh M, Seth P, Dar L, et al. Rapid detection of respiratory viruses by centrifugation enhanced cultures from children with acute lower respiratory tract infections. *J Clin Virol*. 2000;16(1):41–7.
8. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. *Bull World Health Organ*. 2008;86(5):408–16.
9. Steinhoff MC. Acute respiratory infections in children (A new priority for community health workers). *Indian J Community Med*. 1986;11:1–9.
10. Rudan I, Lawn J, Cousens S, Rowe AK, Boschi-Pinto C, Tomašković L, et al. Gaps in policy-relevant information on burden of disease in children: a systematic review. *Lancet*. 2005;365(9476):11–7.
11. Oliveira TF, Freitas GR, Ribeiro LZ, Yokosawa J, Siqueira MM, Portes SA, et al. Prevalence and clinical aspects of respiratory syncytial virus A and B groups in children seen at Hospital de Clínicas of Uberlândia. *Mem Inst Oswaldo Cruz*. 2008;103(5):417–22.
12. Robinson RF. Impact of respiratory syncytial virus in the United States. *Am J Health Syst Pharm*. 2008;65:8–53.
13. Eshaghi A, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, et al. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. *PLoS One*. 2012;7(3):e32807.

14. Anderson LJ, Hierholzer JC, Tsou C, Hendry RM, Fernie BF, Stone Y, et al. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. *J Infect Dis*. 1985;151(4):626–33.
15. Mufson MA, Orvell C, Rafnar B, Norrby E. Two distinct subtypes of human respiratory syncytial virus. *J Gen Virol*. 1985;66(Pt 10):2111–24.
16. Cane PA. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol*. 2001;11(2):103–6.
17. Sullender WM, Mufson MA, Prince GA, Anderson LJ, Wertz GW. Antigenic and genetic diversity among the attachment proteins of group A respiratory syncytial viruses that have caused repeat infections in children. *J Infect Dis*. 1998;178:925–32.
18. Beem M. Repeated infections with respiratory syncytial virus. *J Immunol*. 1967;98:1115–22.
19. Influenza A (H1N1) pandemic 2009 - 2010; 2009. Available from: https://www.who.int/emergencies/situations/influenza-a-(h1n1)-outbreak.
20. WHO. Human infection with pandemic (H1N1) 2009 virus: updated interim WHO guidance on global surveillance; 2009. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2009_08_04-en.
21. Yadav K, Ramachandran VG, Das S, Shah D. Respiratory Syncytial Virus - Detection, Prevalence and Usefulness in Clinical Management: A Hospital based study. *Int J Curr Microbiol App*. 2006;5(2):86–91.
22. Zhang ZY, Du LN, Chen X, Zhao Y, Liu EM, Yang XQ, et al. Genetic variability of respiratory syncytial viruses (RSV) prevalent in Southwestern China from 2006 to 2009: emergence of subgroup B and A RSV as dominant strains. *J Clin Microbiol*. 2010;48(4):1201–7.
23. Mishra P, Nayak L, Das RR, Dwibedi B, Singh A. Viral Agents Causing Acute Respiratory Infections in Children under Five: A Study from Eastern India. *Int J Pediatr*. 2016;2016:7235482.
24. Nikfar R, Shamsizadeh A, Makvandi M, Khoshghalb A. Detection of Respiratory Syncytial Virus in Hospitalized Children with Acute Lower Respiratory Tract Infections, Using RT PCR in Ahvaz. *Arch Pediatr Infect Dis*. 2013;1(3):118–21.
25. Tariq A, Zainab H, Ali B. Detection of Respiratory Syncytial Virus in Hospitalized Children with Acute Respiratory Tract Infections in Kurdistan Region of Iraq. *Glob J Biosci Biotech*. 2016;5(3):335–9.
26. Gupta S, Shamsundar R, Shet A, Srinivasa H. Rapid detection of respiratory syncytial virus (RSV) in children with acute lower respiratory tract infections: a pilot evaluation of an immuno-chromatographic rapid antigen detection method. *Clin Lab*. 2013;59(7-8):929–31.
27. Vardas E, Blaauw D, Mcanerney J. The epidemiology of respiratory syncytial (RSV) infections in South African children. *S Afr Med J*. 1999;89(10):1079–84.

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