Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: February 28, 2023

Citation: Chandy S, Manoharan A, et al., 2023. Respiratory Syncytial Virus among Hospitalised Children in an Era of Pneumococcal Vaccination, Medical Research Archives, [online] 11(2). https://doi.org/10.18103/mra.v11i2.3340

DOI
https://doi.org/10.18103/mra.v11i2.3340

ISSN: 2375-1924

RESEARCH ARTICLE

Respiratory Syncytial Virus among Hospitalised Children in an Era of Pneumococcal Vaccination

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ABSTRACT

Background: Acute respiratory tract infection (ARTI) is the leading cause of paediatric hospitalisations in India, especially ≤5 years of age.

Aim: The study attempts to investigate RSV disease burden in the under-five and any association between NP carriage and viral ARTI.

Methods: This study was conducted from 2019-2020 in Chennai (South India) on hospitalised children with ARTI, between 1 to 60 months of age. Multiplex real-time PCR was employed on nasopharyngeal (NP) samples (n=256) for respiratory pathogens including respiratory syncytial virus (RSV) and *Streptococcus pneumoniae* (SPN).

Results:). Viral and RSV positivity was 81% and 48.6% respectively. Viral co-infections were evidenced in about a quarter of RSV positive children, common being human parechovirus with RSV. Fever, high respiratory rate, severe respiratory distress, cough, difficulty in breathing, chest indrawing and bronchiolitis were significant clinical findings in paediatric viral ARTI. Pneumococcal conjugate vaccine uptake was high (89.4%) in subjects yet 31.87% of vaccinated children had NP pneumococcal carriage. 75% of those with SPN carriage had viral ARTI of which 35.6% were RSV positive. We observed statistically significant association between NP carriage and viral ARTI ($p<0.05$). Antibiotic usage in suspected RSV versus non-RSV viral ARTI was 68% and 93.8% respectively.

Conclusion: Significant RSV disease burden in children < 5 years of age, with ARTI is evidenced with a positive association between SPN carriage and viral acute respiratory tract infections.

Keywords: RSV, Paediatric ARTI, Multiplex real time PCR,

Introduction

Globally, respiratory syncytial virus (RSV) is the most common cause of paediatric lower respiratory tract infections (LRTI), accounting for up to 22% of all acute respiratory tract infections (ARTI), especially in children less than 2 years of age (Bont L et al., 2016; Nair H et al., 2010). RSV infections can present as upper respiratory tract infections (URTIs), bronchiolitis, pneumonia, asthma exacerbations and viral induced wheeze. The highest burden of severe RSV disease necessitating hospitalization, is in infants under 1 year of age. While RSV-associated mortality is higher in immunocompromised children and those with chronic medical conditions, 20% deaths occur in children with no known risk factors.

RSV is a single-stranded, enveloped, unsegmented, negative-sense RNA virus belonging to *Paramyxoviridae* family. Its 15200 nucleotide long RNA codes for 11 structural and non-structural (NS) proteins. The viral envelope contains two important surface glycoproteins: a large glycoprotein (G) and fusion protein (F). Protective immunity is provided by anti-F and G neutralizing antibodies. The main goal of RSV vaccination is to prevent RSV-associated paediatric LRI. Despite the heavy paediatric burden, there is no licensed vaccine for RSV prevention. However, there are more than 60 candidate RSV vaccines in the pipeline targeting pregnant women, neonates, young children and the elderly. (Melero JA et al., 1997).

Streptococcus pneumoniae (SPN) is a respiratory pathogen which may reside asymptomatically in the nasopharynx where it shares an ecological niche with RSV which typically infects the ciliated respiratory epithelium. The interaction between these pathogens has long been the subject of intense research. Secondary pneumococcal infections from increased adherence of SPN to RSV infected cells has been documented, high nasopharyngeal SPN loads reportedly trigger severe RSV infections. The widespread use of PCV has reportedly replaced bacterial pneumonia with RSV ALRI (pneumonia and bronchiolitis) in children below five (Katherine L. O'Brien et al., 2019). Non-vaccine serotype colonisation has also been reported worldwide.

Paediatric RSV has been reported from all parts of India through hospital-based studies done to determine aetiological agents of paediatric ARTI (Mishra P et al., 2016; Mazumdar J et al., 2013; Chodhary ML et al., 2013; Bharaj P et al., 2009; Saxena S et al., 2019; Kini S et al., 2019). Here we present data on the emergence of RSV as a significant paediatric respiratory pathogen in an era of PCV. The association between nasopharyngeal load in young children and susceptibility to viral and RSV infections remains unclear. This study helps assess the possible influence of pneumococcal colonization on RSV and viral ARTI in young children.

Materials

The study was conducted from September 2019 to February 2020 in children up to five years of age. Children hospitalised at Kanchi Kamakoti Childs Trust Hospital (KKCTH), Chennai with WHO criteria for pneumonia with tachypnea or wheeze with or without hypoxia were included. The patients were divided into five groups: Group I (≤ 6 months), Group II (>6 months to ≤1yr), Group III (>1 yr ≤2 yrs) and Group IV (>2 yrs). Those with primary cardiac failure, severe metabolic acidosis without evidence of respiratory tract infection (RTI) were excluded. Informed consent was obtained before enrolment in both the studies and all relevant clinical data captured prospectively in a study-specific case report form (CRF).

Methods

Nasopharyngeal (NP) swab was collected from enrolled subjects in viral transport medium (VTM). The study used Fast Track Diagnostics (FTD, Junglinster, Luxembourg) Respiratory Pathogens 21 plus kit which can detect 20 viral and 5 bacterial pathogens including influenza A virus (IFV-A), influenza A(H1N1) swl, influenza B (IFV-B), coronaviruses (NL63, 229E, OC43 and HKU1), parainfluenza viruses types 1 to 4 (PIV1-4), human metapneumovirus A and B (hMPV), rhinovirus (RV), respiratory syncytial (viruses A and B (RSV), adenovirus (hAdV), enterovirus (EV), human parechovirus (HPeV), human bocavirus (HBoV), *M. pneumoniae*, *C. pneumoniae*, *S. pneumoniae*, *H. influenzae* B and *S. aureus*. Real time PCR was done on QuantStudio 5 Dx (Thermo Fisher Scientific ®). All testing was conducted at The Childs Trust Medical Research Foundation-Molecular Laboratory (CTMRF-ML) as per the manufacturer's instructions. An internal control (IC) was used to validate the assays. A Ct≤ 33 was considered positive for all targets. Ct Values were correlated with viral load as per **Table 1**.

Table 1: Correlation of Ct values and viral load

Ct value	Score
<=25	High viral load
>25 ≤ 30	Moderate viral load
>30 ≤ 33	Low viral load

Statistical Analysis

Statistical analysis done by the statistical software STATA 11.0. All categorical variables were represented as percentages. The association between categorical variables was studied by

McNemar's test. The p value of <0.05 will be considered as significant.

Results

Two hundred and fifty-six patients (n=256) up to the age of 5, were recruited in the study which included males (n=170, 66.4%) and females (n=86, 33.5 %). About half the subjects (n=148, 57.8%) were at least a year old and more than three fourths (n= 204, 79.6%) up to two years of age. 60% of subjects were from Chennai, followed by those from Andhra Pradesh (19%), a neighbouring state. Viral positivity was high from September to November while RSV infections were significantly high during September to October (northeast monsoon) and declined thereafter. Overall, 188 (73.4%) enrolled subjects had evidence of viral respiratory tract infection of which 48.4% (n=91) were RSV positive (Table 2)

Viral positivity and RSV positivity was; Group I (79.7% and 51%), Group II (82.8% and 45.3%), Group III (73.3% and 23.2%) and Group IV (51.9% and 11.5%). A quarter of the RSV positives (n=23, 25.2%) had co-infections predominantly with, human parechovirus (HPeV, 43.4%). Ct values were inversely proportional to viral load with high Ct values indicating lower viral load and low Ct values, a higher viral load (**Table 2 and Table 3**)

Table 2: Viral infections and SPN carriage amongst different age groups

S. no	Age	No of subjects (n=256)	Male (n=170	Female (n=86)	Viral positives (n=188)	RSV positives (n=91)	SPN positives (n=87)
1	≤ 6 Months	84	54	30	67	43	18
2	>6 Months to ≤1Years	64	46	18	53	29	22
3	**≤ 1 Years**	**148**	**100**	**48**	**120**	**72**	**40**
4	> 1 Years ≤2 Years	56	39	17	41	13	25
5	**≤ 2 Years**	**204**	**139**	**65**	**161**	**85**	**65**
6	> 2 Years to 5 Years	52	31	21	27	6	22

Table 3: RSV load amongst different age groups

S. no	Age	RSV≤25	RSV >25 ≤ 30	RSV >30 ≤ 33
1	≤ 6 Months	30	8	5
2	>6 Months to ≤1Years	17	9	3
3	> 1 Years ≤2 Years	8	5	0
4	>2 Years to 5 Years	5	1	0
	Total	60 (65.9%)	23	8

There was no significant difference in the mean Ct of RSV mono-infections and co-infections. Fever, high respiratory rate, severe respiratory distress, cough, difficulty in breathing and chest indrawing were clinical features seen in 95% of viral and RSV positives. Lethargy and inability to drink were seen in about three-fourths of the confirmed cases. There was no mortality in the study. 68% of confirmed-RSV and 93.8% of the non-RSV viral cases received antibiotics prior to admission. Mean days of hospitalisation for confirmed-RSV and non-RSV viral cases was 4.2 and 5 days respectively. (**Table 4**)

Table 4: Clinical features of viral ARTI cases

Signs and Symptoms	All viral positives (n=188)	RSV positives (n=91)
Fever	188 (100%)	91 (100%)
Respiratory Rate	187 (99.4%)	90 (98.9%)
Severe respiratory distress	185 (98.4%)	88 (96.7%)
Unable to drink	159 (84.5%)	81 (89%)
Vomiting	82 (43.6%)	58 (63.7%)
Convulsions	1 (0.53%)	0
Lethargy	179(95.2%)	89 (97.8%)
Irritability	171 (90.9%)	81 (89%)
X ray confirmation of pneumonia	51 (27.1%)	15 (16.4%)
Stridor in a calm child	12 (6.3%)	3 (3.2%)
Antibiotics prior to admission	153 (81%)	62 (68.1%)
PCV vaccine	174 (92.5%)	87 (95.6%)
Cough	187 (99.4%)	91 (100%)
Difficulty breathing	187(99.4%)	90 (98.9%)
Cheat Indrawing	187(99.4%)	90 (98.9%)
Severe malnutrition	0	0
Antibiotics prescribed at discharge	76 (40.4%)	23 (25.2%)

87 (33.9%) subjects showed evidence of nasopharyngeal SPN carriage. Of these, 45.9% carriage occurred in children ≤1 year of age and 54% in older children (>1 year ≤5 years). Moderate to high SPN load was seen in 60% of those with nasopharyngeal carriage. There was a positive association between SPN nasopharyngeal carriage and viral ARTI; 74.7%(n=65) of those with SPN nasopharyngeal carriage had viral ARTI. Viral ARTI was largely evidenced amongst patients with high to moderate SPN load. PCV vaccination was high (89.4%) in enrolled subjects. Nasopharyngeal SPN carriage was seen in 31.87% of vaccinated children and 51.8% unvaccinated children.

Discussion

RSV is the commonest cause of LRI in infants below 24 months of age in India. Those at the highest risk of RSV related LRI reportedly are infants between 1 month to 12 months of age with infants between 6 weeks and 6 months of age experiencing highest rates of infection. Over 95% of children are infected with RSV by 2 years of age. (Mishra P et al., 2016; Mazumdar J et al., 2013; Chodhary ML et al., 2013; Bharaj P et al., 2009; Saxena S et al., 2019; Kini et al., 2019). In young children, the lumen of the bronchioles in the airway is narrow and prone to obstruction as in case of infection. Increased airway resistance presents as wheeze and croup. RSV-related viral bronchiolitis in infancy is reportedly an antecedent for recurrent wheezing, airway hyper-responsiveness and asthma during childhood (Shi T et al., 2017). In our study, majority of children presenting with ARTI requiring hospitalization were below 2 years of age. Our results resonate with results of previous studies in India, with 79% of RSV infections being evidenced in children up to one year of age.

Most studies on paediatric ARTI have reported seasonality of RSV disease. Generally, RSV infections correlate with cold and humid weather conditions. In northern and western parts of India, all RSV types have been detected during winter months and post-monsoon season with peaks in December and January (Kini S et al., 2019; Broor S, et al., 2018). A study from Jaipur, has documented year-round RSV circulation with a peak at the end of winter season in March (Swamy MA, et al 2017). In

Odisha, RSV peaks during rainy season (June to September) and then during the winter months (December to January). In Kolkata, the predominant RSV season seems to be from November to February. In South India, RSV circulation coincides with the onset of the rainy season through November with a small spike during January and February. Mixed RSV infections may occur throughout the year (Kini S et al., 2019; Broor S et al., 2018).

Multiplex real time RT-PCR is an excellent tool for RSV epidemiology studies. Its high sensitivity can detect small amounts of nucleic acids of multiple pathogens in the same sample. Currently, commercially available real time multiplex PCR kits can detect a large number of respiratory pathogens, including bacteria, fungi, viruses and parasites from a single sample. Detection of multiple viruses should be interpreted with caution as children shed RSV and other respiratory viruses at higher titres for a longer time than adults. Real time multiplex PCR has enabled simultaneous detection of multiple respiratory viruses. Co-infections of RSV with other respiratory viruses and mixed infections of RSV A and RSV B has widely been reported. Mixed viral infections were reportedly seen in 30% of wheezing cases in the under-five and in 2.5-5% of paediatric RSV cases (Choudhary ML et al., 2013; Bharaj P et al., 2009; Saxena S et al., 2019; Swamy MA et al., 2017).

The diagnostic significance of multiple pathogens in paediatric LRTI and the severity and clinical outcome of single RSV infections versus coinfections is unclear. There are conflicting reports on clinical severity of RSV co-infections; while some studies have shown to have less severe clinical impact, others have documented severe clinical phenotypes in RSV associated co-infections. Clinical severity of coinfections of non-RSV viruses is similar to that of single infections; while coinfections involving RSV are more severe resulting from a reduced immune response (Brand HK., et al 2012).

Clinical manifestations of RSV infection can range from URI to LRI. It is significantly associated with paediatric bronchiolitis and pneumonia. RSV is less cytopathogenic than other respiratory viruses, the damage caused in the respiratory airway during RSV infection may be due to the upregulated immune response rather than the replicating virus per se (Griffiths C., et al 2017).

S.pneumoniae and RSV are often detected simultaneously in infants hospitalized with bronchiolitis or pneumonia. Higher nasopharyngeal SPN loads are seen in viral pneumonia, mainly in those of RSV aetiology (Madhi SA et al., 2004). Our results indicate that high loads of SPN may predispose to paediatric viral infections. The role of SPN in viral ARTI has been the subject of intense research and studies which report that colonization with SPN increases the risk of viral respiratory infections or augments symptoms. (Madhi SA et al., 2004; Dagan R et al., 2001; Morpeth SC et al., 2018)

PCV is expected to reduce the incidence of paediatric pneumococcal infections in India. Nasopharyngeal SPN surveillance in children can indicate the impact of the PCV on vaccine-type carriage and emergence of non-vaccine serotypes. In our study, SPN colonisation between PCV vaccinated and PCV unvaccinated subjects was 31.8% and 51.8% respectively. This could indicate possible serotype replacement by non-vaccine types which can reduce protection afforded by PCV.

Ct (cycle threshold) values are a semi-quantitative measure of the load of viral genetic material in patients' samples. Ct values are inversely proportional to the viral genetic load. We have attempted use of Ct values to understand viral and SPN loads. Our results indicate a high RSV load during the acute phase of ARTI in children. This could have implications in the transmission of RSV infections.

Despite its impact worldwide, there are very few options for treatment and prevention of RSV infections. In India, RSV is a significant cause of hospitalization in the under-five. While we await an efficacious RSV vaccine, an active surveillance program through simple bedside point of care (POC) devices and laboratory testing will help improve clinical management, reduce nosocomial transmission and unnecessary antibiotic usage.

Conflict of Interest- The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Source- The study was funded through Intramural Research Grant of The CHILDS Trust Medical Research Foundation.

Ethical Approval Statement- The study was approved by the Institutional Ethics Committee vide KKCTH-CTMRF IEC-11/October 2017(IRB min.dt.25.10.2017).

Acknowledgements: The authors would like to thank Department of Paediatrics, KKCTH for contributing the clinical cases. Mr Abdul Hameed (CTMRF-ML) and Mr Robinson.P (CTMRF-ML) are thanked for technical assistance and data entry.

References

1. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. *Pediatr Infect Dis J*. 2005;24(7):605-610. doi:10.1097/01.inf.0000168741.59747.2
2. Bharaj P, Sullender WM, Kabra SK, et al. Respiratory viral infections detected by multiplex PCR among pediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. *Virol J*. 2009;6:89. Published 2009 Jun 26. doi:10.1186/1743-422X-6-89
3. Bont L, Checchia PA, Fauroux B, et al. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. *Infect Dis Ther*. 2016;5(3):271-298. doi:10.1007/s40121-016-0123-0
4. Brand HK, de Groot R, Galama JM, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. *Pediatr Pulmonol*. 2012;47(4):393-400. doi:10.1002/ppul.21552
5. Broor S, Parveen S, Maheshwari M. Respiratory syncytial virus infections in India: Epidemiology and need for vaccine. *Indian J Med Microbiol*. 2018;36(4):458-464. doi:10.4103/ijmm.IJMM_19_5
6. Choudhary ML, Anand SP, Wadhwa BS, Chadha MS. Genetic variability of human respiratory syncytial virus in Pune, Western India. *Infect Genet Evol*. 2013;20:369-377. doi:10.1016/j.meegid.2013.09.025
7. Dagan R, Sikuler-Cohen M, Zamir O, Janco J, Givon-Lavi N, Fraser D. Effect of a conjugate pneumococcal vaccine on the occurrence of respiratory infections and antibiotic use in day-care center attendees. *Pediatr Infect Dis J*. 2001;20(10):951-958. doi:10.1097/00006454-200110000-00008
8. Griffiths C, Drews SJ, Marchant DJ. Respiratory Syncytial Virus: Infection, Detection, and New Options for Prevention and Treatment. *Clin Microbiol Rev*. 2017;30(1):277-319. doi:10.1128/CMR.00010-16
9. Kini S, Kalal BS, Chandy S, Shamsundar R, Shet A. Prevalence of respiratory syncytial virus infection among children hospitalized with acute lower respiratory tract infections in Southern India. *World J Clin Pediatr*. 2019;8(2):33-42. Published 2019 Apr 9. doi:10.5409/wjcp.v8.i2.33
10. Madhi SA, Klugman KP; Vaccine Trialist Group. A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nat Med*. 2004;10(8):811-813. doi:10.1038/nm1077
11. Mazumdar J, Chawla-Sarkar M, Rajendran K, et al. Burden of respiratory tract infections among paediatric in and out-patient units during 2010-11. *Eur Rev Med Pharmacol Sci*. 2013;17(6):802-808.
12. Melero JA, García-Barreno B, Martínez I, Pringle CR, Cane PA. Antigenic structure, evolution and immunobiology of human respiratory syncytial virus attachment (G) protein. *J Gen Virol*. 1997;78 (Pt 10):2411-2418. doi:10.1099/0022-1317-78-10-2411
13. 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. doi:10.1155/2016/7235482
14. Morpeth SC, Munywoki P, Hammitt LL, et al. Impact of viral upper respiratory tract infection on the concentration of nasopharyngeal pneumococcal carriage among Kenyan children. Sci Rep. 2018;8(1):11030. Published 2018 Jul 23. doi:10.1038/s41598-018-29119-w
15. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet*. 2010;375(9725):1545-1555. doi:10.1016/S0140-6736(10)60206-1.
16. Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study [published correction appears in Lancet. 2019 Aug 31;394(10200):736]. *Lancet*. 2019;394(10200):757-779. doi:10.1016/S0140-6736(19)30721-4
17. Saxena S, Tripathi R, Kushwaha RAS, Kumari R, Bhattacharya P and Dhole TN: Comparison of conventional and real time PCR for monitoring of respiratory syncytial virus among pediatric patients in Northern India 2011-2014. Int J Pharm Sci & Res 2019; 10(5): 2294- 00. doi: 10.13040/IJPSR.0975-8232.10(5).2294-00.
18. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and

modelling study. *Lancet*. 2017;390(10098):946-958. doi:10.1016/S0140-6736(17)30938-8

19. Swamy MA, Malhotra B, Reddy PV, Tiwari JK, Kumar N, Gupta ML. Trends of respiratory syncytial virus sub-types in children hospitalised at a tertiary care centre in Jaipur during 2012-2014. *Indian J Med Microbiol*. 2017;35(1):134-136. doi:10.4103/ijmm.IJMM_15_306
20. Zar HJ, Polack FP. Childhood pneumonia: the role of viruses. *Thorax*. 2015;70(9):811-812. doi:10.1136/thoraxjnl-2015-207320