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Original Article

Respiratory syncytial virus and human metapneumovirus in severe lower respiratory tract infections in children under two

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Abstract

Introduction: Viruses are the most important causative agents of acute lower respiratory tract infections (ALRTIs), ranked as the second leading cause of death and the primary cause of hospitalization in children. Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are among the commonest viral causes of severe ALRTI. In this study, we aimed to study the burden of both RSV and hMPV in causing severe ALRTI in children younger than two years of age admitted to the pediatric intensive care unit (PICU).

Methodology: Nasopharyngeal swabs were collected from children admitted to the PICU with a diagnosis of community-acquired ALRTI who were two years of age or younger. Real-time polymerase chain reaction (RT-PCR) was used to test for RSV and hMPV.

Results: A total of 127 swabs were screened for RSV and hMPV, of which 49.6% were negative for RSV and hMPV, 46.4% were positive for RSV, and 3.9% were positive for hMPV. With respect to RSV, the mean age of cases (4.01 ± 5.05) and the monthly distribution (mainly January) were the most important risk factors. There were no statistically significant differences between the RSV group and control group regarding duration of hospital stay, mechanical ventilation need or duration, and underlying chronic conditions.

Conclusions: RSV is important viral cause of severe ALRTIs in children younger than two years of age during this study period; hMPV played a minor role.

Key words: lower respiratory tract infections; respiratory syncytial virus; human metapneumovirus; pediatric ICU.

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Introduction

With a wide range of clinical presentations, respiratory tract infections remain a significant cause of morbidity and mortality worldwide, particularly in children [1]. Respiratory infections represent a major public health problem because of their high incidence and ease of spread in the community [2].

The World Health Organization (WHO) has initiated a program for clinical management and control of acute respiratory tract infections (ARTIs), which has resulted in the reduction of ARTI mortality rates by 25% to 67% [3]. ARTIs represent 30%–50% of pediatric medical admissions [4]. Pediatric patients with either upper or lower respiratory tract infections are typically treated symptomatically as outpatients [5]. Although many pathogens may cause respiratory tract infections, viruses are the most frequently implicated [1]. Viral pathogens are responsible for 30%–40% of RTIs [4]. The etiology of the majority of lower respiratory tract infections (LRTI) is thought to be viral, yet in only 40% of cases can a viral agent be identified. These observations suggest that unknown pathogens may be responsible for a substantial proportion of respiratory tract diseases [6]. The impact on the severity of early life respiratory infections may be also affected by viral types diagnosed through sensitive polymerase chain reaction (PCR) analyses. Some studies have shown a positive association between viral types and worse clinical outcomes, while others have failed to show results in the same direction [7]. Respiratory syncytial virus (RSV) is the main cause of LRTIs in young children in both developed and developing countries, and almost 34 million new cases occur every year worldwide [8]. In the developing world, RSV accounts for 3.4 million hospitalizations for LRTIs in children under five years of age [8] and it is considered the most common cause of serious lower respiratory infection in infants [5]. The viral etiology represents about 59.9% of cases of ALRTI in children five years of age and younger in Egypt. A previous study showed that RSV was found in 23.8% of cases, with 34.8% of the children under six months of age. Compared to other

tested viruses, RSV was significantly more common in this age group [5]. A predominance of RSV and human metapneumovirus (hMPV) was observed in Brazil and in other countries [9]. While the role of respiratory viruses including hMPV and RSV is well established in causing self-limiting upper respiratory tract infections or mild pneumonia, their contribution to causing severe and radiologically proven pneumonia in developing countries is less clear [10]. The aim of this study was to determine the prevalence and disease severity of RSV and hMPV PCR-positive infants two years of age and younger with severe ALRTI.

Methodology

This cross-sectional study was conducted between December 2013 and May 2014 on children diagnosed with ALRTI admitted to the pediatric ICU of the Children's Hospital of Cairo University.

The study population consisted of children two years of age or younger hospitalized during these six months with a diagnosis of (a) severe community-acquired ALRTI, (b) severe bronchiolitis or pneumonia according to the WHO's definition (c) tachypnea (respiratory rate of 60 breaths per minute in children < 2 months, 50 breaths per minute in children 2–12 months, and > 40 breaths per minute in children > 12 months of age), (d) chest indrawing, or (e) any other danger sign (grunting, persistent vomiting, convulsions or unconsciousness or both), proven by positive radiological findings and requiring ICU admission. The study excluded children with nosocomial LRTIs and those with isolated non-respiratory causes of respiratory distress.

All cases were subjected to full history (age, sex, presenting symptoms, onset and course of the disease, and estimation of risk factors and co-morbidities). Full data on general and local examination of the child, admission laboratory and radiological investigations, need for oxygen therapy or mechanical ventilation, secondary complications, use of antibiotics, detection of bacterial co-infection detected by sputum culture on admission, duration of hospitalization, readmission, and outcome were recorded. Respiratory viral detection was done by nasopharyngeal swabs on admission.

Approval from the research committee of the pediatric unit at Cairo University was obtained. Formal consent was obtained from the caregivers of the participants who agreed to their child's participation in the study.

Viral detection/PCR analysis

Nasopharyngeal (NP) swabs (UTM Kit, COPAN Italia, Brescia, Italy), were obtained, transported, and preserved on viral transport media (Hank's balanced salt solution, Gibco, Invitrogen, NY, USA) with 2.5% w/v bovine serum albumin (Sigma, California USA), 2% penicillin/streptomycin (Gibco, Invitrogen,), and 2.5% HEPES Buffer (Gibco, Invitrogen) was added to each aspirate [11]. The received swabs inside the 15 mL tube were agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip.

Viral testing was done by real-time multiplex PCR using AnyplexTMII RV16 Detection (V1.1) supplied by Seegene, Seoul, South Korea, operated on CFX96TM Real-time PCR System (Bio-Rad, Berkeley, USA). Nucleic acid extraction was done automatically using SEEPREP12 Viral supplied by NorDiag (NorDiag, Oslo Norway), using the extraction Seeprep machine (Seegene). Protocol viral RNA was operated using 530 uL from the sample to result in an eluted volume of 60 uL. Reverse transcription was done using cDNA synthesis kit for manual set up cDNA Synthesis Premix (SGRT801) from Seegene. Interpretation of the results was done automatically using the Seegene viewer software after exporting the run data to it.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Comparison between mean values of different variables in the two studied groups was performed using the unpaired t test. Comparison between categorical data was performed using the Chi-square test. Correlation between onset of disease and viral load detected by PCR was performed using Spearman's rho correlation coefficient test. To obtain the total value of the odds ratio (OR) and to determine the predictors for RSV disease, logistic regression analysis was applied. SPSS software version 16 was used for data analysis. P value less than or equal to 0.05 was considered significant and < 0.01 was considered highly significant.

Results

The present study included 127 infants with severe LRTI; 52% males and 48% females. The viral research results, based on the PCR method, were negative for RSV and hMPV in 63 patients (49.6%), positive for RSV in 59 patients (46.4%) with a mean age of 4 months, and positive for hMPV in 5 patients (3.9%) with mean age 6.6 months. Most RSV cases occurred during January, while two out of five cases of hMPV were detected during April. Co-infection of the two

viruses studied was not observed in any of the positive patients. RSV subtypes were also assessed, and it was found that RSV type B was the predominant type in the patients (78%) while RSV type A was found in 18.6% of patients, with two cases (3.4%) of co-infection with both types of the virus. In the RSV-positive patients, 44.4% had a + load, 46.03% had ++ load, 6.3% had +++, load but there was no statistically significant correlation between onset of the respiratory illness and the viral load in RSV-positive patients ($r = 0.053$; $p = 0.696$).

As the primary goal of the study was to estimate the role of RSV and hMPV in causing severe LRTI in children under two years of age, and since the number of patients with isolated hMPV was low, which made it difficult to reach solid conclusions about the disease severity, cases with causes other than hMPV or RSV were considered as the control group and compared with the RSV-positive group for the additional analyses for statistical purposes.

The admission diagnoses to the PICU depending on the chest X-ray finding of each group were categorized, and it was determined that, in the RSV-positive group, 59.3% of patient were diagnosed with pneumonia, 25.4% were diagnosed with bronchiolitis, and 15.3% were diagnosed with both pneumonia plus bronchiolitis with no statistically significant difference between both groups ($p > 0.05$) (Table 1).

In the hMPV-positive group, two cases were admitted with pneumonia, one diagnosed with bronchiolitis, one with asthma exacerbation, and one case with pneumonia plus bronchiolitis.

Regarding monthly distribution, the RSV-positive patients showed highly significant statistical difference from that of the negative group, with most of cases detected during the month of January (26 cases) (44.1%), while in April, there was only 1 case (1.7%) ($p = 0.001$, OR = 2.551, 95% CI = 1.766–3.686) (Table 2). Also, there was a statistically significant difference regarding the mean age between both groups; the mean

Table 1. Admission diagnoses of the RSV and non-RSV/hMPV groups

Admission diagnosis	RSV group (n = 59)	Non- RSV/hMPV group (n = 63)	P value
Pneumonia (n = 74)	35 (59.3%)	39 (61.9%)	0.475
Bronchiolitis (n = 33)	15 (25.4%)	18 (28.6%)	
Asthma exacerbation (n = 1)	0 (0%)	1 (1.6%)	
Pneumonia + bronchiolitis (n = 14)	9 (15.3%)	5 (7.9%)	

RSV: respiratory syncytial virus; hMPV: human metapneumovirus; Data are expressed as number (%).

Table 2. Demographic data of RSV and non-RSV/hMPV groups.

	RSV group (n = 59)	Non RSV/hMPV group (n = 63)	95% CI	OR	P value
Gender					
Male	28 (47.5%)	34 (54%)	0.644–2.937	1.375	0.472
Female	31 (52.5%)	29 (46%)			
Age	4.01 ± 5.05	7.03 ± 6.23	0.831–0.964	0.895	0.004
Monthly distribution					
December (n = 17)	9 (15.3%)	8 (12.7%)	1.766–3.686	2.551	0.001
January (n = 39)	26 (44.1%)	13 (20.6%)			
February (n = 20)	17 (28.8%)	3 (4.8%)			
March (n = 35)	6 (10.2%)	29 (46.0%)			
April (n = 11)	1 (1.7%)	10 (15.9%)			

RSV: respiratory syncytial virus; hMPV: human metapneumovirus; Data are expressed as number (%).

Table 3. Relationships between both groups of co-morbidities on admission**.**

Co-morbidities	RSV group n = 59 (%)	Non-RSV and hMPV group n = 63 (%)	P value
Coronary heart disease (n = 26)	11 (18.6%)	15 (23.8%)	0.486
Down syndrome (n = 7)	3 (5.1%)	4 (6.3%)	0.764
Neurological (n = 14)	7 (11.9%)	7 (11.1%)	0.896
Respiratory (n = 10)	3 (5.1%)	7 (11.1%)	0.225
Previous admission (n = 25)	9 (15.3%)	16 (25.4%)	0.165
Bacterial co-infection (n = 31)	15 (25.4%)	16 (25.8%)	0.962

RSV: respiratory syncytial virus; hMPV: human metapneumovirus; Data are expressed as number (%).

age of the RSV-positive patients was 4.01 ± 5.05 compared to 7.03 ± 6.23 in the other group (p = 0.004, OR = 0.860, 95% CI = 0.787–0.939).

The proportion of children with underlying medical disorders was estimated across groups and compared between RSV-positive patients and control groups, and there was no statistically significant difference between both groups (Table 3). The bacterial co-infection on admission was variable and included *Streptococcus pneumoniae* (3.4% of the RSV group and 3.2% of the non-RSV/hMPV group), *Klebsiella pneumoniae* (10.1% of the RSV group and 14.2% of the non-RSV/hMPV group), *Pseudomonas aeruginosa* (3% of the RSV group and 3.2% of the non-RSV/hMPV group), *Enterobacter* (1.7% of the RSV group and 1.6% of the non-RSV/hMPV group), *Acinetobacter baumannii* (1.7% of the RSV group and 3.2% of the non-RSV/hMPV group), *Escherichia coli* (3.4% of the RSV group) and coagulase-negative *Staphylococci* in 1.6% of the non-RSV/hMPV group.

Comparing disease severity and outcome between the RSV-positive and non-RSV/hMPV groups (Table 4), there was no statistically significant relationship between the two groups. Of the RSV-positive group, 49.2% of patients were ventilated during their PICU stay. The median length of stay in the PICU for the RSV-positive cases was 11.93 ± 10.47 days. Fifteen percent of the RSV-positive group resulted in death. On assessment of various complications that were suffered by the RSV-positive patients during their PICU stay such as secondary sepsis, heart failure, convulsions, and pneumothorax , there was a statistically significant relationship between the development of pneumothorax and RSV in comparison with the non-RSV/hMPV group (p = 0.01, OR = 9.725, 95% CI = 1.177–80.348), as 13.6% of RSV-positive cases (eight cases) suffered from pneumothorax compared with only 1.6% (one case) in the non-RSV/hMPV group (Table 4). With respect to disease severity, the two subtypes of the RSV (subtype A and subtype B) were compared; RSV type B was the predominant subtype detected in the patients, but there was no statistically significant difference in the compared parameters (Table 5). Unfortunately, the small number (five) of positive cases for hMPV did not allow for statistical detailed analysis and comparison of clinical aspects and outcomes between the hMPV group and the other two groups.

Discussion

Acute respiratory tract infections are among the most common causes of mortality among young children worldwide and the most common cause of acute respiratory failure in PICUs among children younger than five years of age [12]. Determining the etiology of LRTIs in children has long been of interest to the research and clinical community. Viruses have been shown to be the causative agent in 36%–85% of LRTIs among children [5]. The most common cause of acute respiratory tract infections in young children is RSV. However, hMPV, first described in 2001, is a frequent cause of acute respiratory tract infections, requiring PICU admission in young children [12]. In the present study, the viral assay results, based on the PCR method, had 59 patients (46.4%) positive for RSV, 5

Table 4. Relationship between both groups regarding disease severity, outcome, and secondary complications.

	Non-RSV and hMPV group (n = 63)	RSV group (n = 59)	P value
O2 therapy duration (days)	15.46 ± 18.92	11.76 ± 10.56	0.189
MV need	37 (58.7%)	29 (49.2%)	0.290
MV duration (days)	8.90 ± 18.99	6.80 ± 10.66	0.455
Length of stay (days)	15.46 ± 18.92	11.93 ± 10.47	0.209
Morality	18 (28.6%)	9 (15.3%)	0.081
Sepsis (n = 16)	8 (13.6%)	8 (12.7%)	0.888
Heart failure (n = 6)	3 (5.1%)	3 (4.8%)	0.934
Convulsions (n = 5)	3 (5.1%)	2 (3.2%)	0.595
Pneumothorax (n = 9)	8 (13.6%)	1 (1.6%)	0.011

RSV: respiratory syncytial virus; hMPV: human metapneumovirus; MV: mechanical ventilation; Data are expressed as mean ± SD or number (%).

Table 5. Comparison of disease severity between RSV subtypes.

	RSV subtype A (n = 10)	RSV subtype B (n = 47)	P value
MV need	5 (50.0%)	23 (50.0%)	1.000
LOS (days)	12.60 ± 12.11	11.35 ± 9.58	0.722
Mortality	2 (20.0%)	6 (13.0%)	0.569

RSV: respiratory syncytial virus; MV: mechanical ventilation; LOS: length of stay; Data are expressed as number (%).

patients (3.9%) positive for hMPV, and 63 patients (49.6%) negative for RSV/hMPV, and no cases of co-infection with the two viruses. The proportion of patients positive for RSV was similar to that in a previous study that found 48% of children were infected with RSV alone; on the other hand, that study revealed that 17% of cases were infected with hMPV alone [13]. Substantial variation in the timing of community outbreaks of RSV disease from year to year exists within and between communities in the same year, even in the same region [14]. In a study conducted in Egypt by Shafik *et al*. [5] aiming to identify the relative prevalence of various respiratory viruses that contribute to LRTIs in young children under five years of age, RSV was determined to be the predominant viral agent among children with LRTI using RT-PCR; also, RSV could only be detected from November through mid-February. Another study by McGuiness *et al*. [15] estimating the RSV season in the United States by the Centers for Disease Control and Prevention found that the RSV season onset occurred in October/November of each year with an offset occurring in March/April of the following year. Also, Leung *et al*. [16] found that RSV-associated PICU admissions in Hong Kong appeared to follow the overall pattern of RSV outbreaks, which usually begin in November or December, peak in January or February, and end by the end of March or sometime in April. In our study, RSV was by far the predominant virus detected, with infections detected throughout the study period (from December until April), with peaks during the months of January (44.1%) and February (28.8%). Our data coincide with that of another study conducted in Egypt by Fattouh *et al*. [17], where most RSV cases (97.1% of cases) occurred mainly between December and February, which are the cold months of the year in Egypt. In the present study, RSV subtype B was predominant (78%) in our PICU patients compared to 18.6% infected with subtype A, and 3.4% with co-infection by both types. When comparing the severity of the disease produced by each subtype, we found no statistically significant difference between the two groups with respect to the presenting symptoms, need for oxygen and mechanical ventilation, or mortality. Published reports that have compared the two RSV subtypes in terms of disease severity produced controversial results; studies by Walsh *et al*. [18] and Papadopoulos *et al*. [19] found that RSV subtype A infection resulted in more severe disease in hospitalized infants, but a study by Hornsleth *et al*. [19] revealed that RSV type B infection produced more severe disease than RSV type A in terms of length of hospital stay, use of respiratory support, and the presence of an infiltrate by chest radiograph. Also, Cintra *et al*. [20] revealed that RSV subtypes A and B were co-circulating within the same time period in children seen at the emergency department, with varying predominance of either subgroup, and no significant association of RSV subgroup with disease severity, but only a trend of RSV subgroup B being more frequent in children with risk factors for severe disease.

The present study showed a significant statistical difference in age distribution between the RSV patients and the control group as the mean age of RSV patients was 4.01 ± 5.05, while the control group was 7.03 ± 6.23 ($p = 0.004$). Young age is a known vulnerability for severe RSV infection, and that was demonstrated in many studies [17,21-23]. This can be attributed to lower cellular immunity [24], as in infants younger than six months of age, the maternally acquired antibodies are decreasing, with a half-life of about one month associated with lower magnitude of the humoral immune response to RSV in children younger than three months of age [25].

In our study, 49.2% of patients in the RSV group required mechanical ventilation support. In Zhang *et al*.'s [21] study, estimating 171 cases with PICU admission due to RSV severe pneumonia, only 22% (37/171) required assisted ventilation support; however, the researchers excluded children with underlying chronic conditions, which this may explain the difference in the percentage of patients requiring mechanical ventilation. Mechanical ventilation for patients with bronchiolitis is not an easy issue. The small airway obstruction and inflamed edematous alveoli makes the lungs very prone to exaggerated air trapping and barotraumas, which will further cause air leaks in the lungs such as pneumothorax and pneumo-mediastinum. In our study, pneumothorax was significantly the most frequently encountered complication (13.6%) with RSV infection ($p = 0.01$). Strong positive correlation between the incidence of air leaks and high ventilatory pressure or large tidal volume was found by Briassoults *et al*. [27]. Hui *et al*. [26] reported a case of life-threatening bilateral pneumothorax in an infant with severe bronchiolitis, while Odek *et al*. [28] and Pollack *et al*. [29] each reported a case of spontaneous bilateral pneumothorax in an infant with RSV bronchiolitis. However, since only 10 patients developed pneumothorax in the present study, a type 1 error could not be excluded.

Our results indicate a low incidence of hMPV infections in children with severe ALRTI during the winter period of 2013–2014. This finding is consistent

with data showing that hMPV has both seasonal and annual distribution; in a study conducted in Italy by Caracciolo *et al*. [30], the incidence of hMPV infection was 25.3% during the 2005–2006 winter-spring season, whereas a much lower rate of infection (4.7%) was found during the following 2006–2007 winter season. In a study by Manoha *et al*. [31] conducted among French children, hMPV was detected in 10.1% of children during the 2002–2003 winter season and in 3.3% of children during the 2003–2004 winter season.

There are several limitations to our study. The seasonality of infection with RSV or hMPV could bias our results, as our study took place over 6 months only, whereas a study of a full 12 months may have given a more accurate representation of the incidence of infection with RSV or hMPV. Another weakness of this study was the fact that only patients with infections severe enough to be hospitalized were included. Therefore, the prevalence of hMPV- and RSV-induced LRTIs in our study population may underestimate their respective prevalence in the community.

Conclusions

Our data reveals that RSV is an important viral cause of severe LRTI requiring PICU admission in children younger than two years of age. An age distribution analysis showed RSV infections occurred in significantly younger patients with peak during the month of January and February, so RSV vaccination or prophylaxis, once available, may offer considerable public health benefit for vulnerable populations in this season. Finally, our findings provide evidence of the substantial contribution of RSV to the global burden of ALTRIs. Further studies are required to accurately define the risk groups who are in need of immunoprophylaxis. Analyses of hMPV infections are required over successive seasons to prove the difference in the burden of its infection on a yearly basis and to detect reasons for that phenomenon if proven.

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