CAUSATIVE AGENTS OF SEVERE COMMUNITY ACQUIRED VIRAL PNEUMONIA AMONG CHILDREN IN EASTERN THAILAND

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Abstract. Pneumonia is a leading cause of morbidity and mortality among infants and young children. The most common causes of pneumonia in children are respiratory viruses. In Thailand, the epidemiology of the viruses causing community-acquired pneumonia (CAP) among children is poorly defined. In this cross sectional study we used nasopharyngeal samples collected from hospitalized children diagnosed with severe CAP in accordance with WHO criteria between June 2013 and May 2014 to determine the causes of infection. The samples were analyzed for respiratory syncytial virus (RSV), parainfluenza viruses (PIV) types 1,2 and 3, adenovirus, rhinovirus, influenza viruses types A and B and coronavirus by polymerase chain reaction (PCR) and reverse transcriptase-polymerase chain reaction (RT-PCR). Of 102 cases of severe CAP, samples were obtained in 91 cases and 48 (52.7%) were positive for respiratory viruses. The most common viruses were RSV (n=22; 45.8%), rhinovirus (n=11; 22.9%) and adenovirus (n=9; 18.7%). Patients were aged 1 month to 4 years 5 months, with a median age of 1 year 1 month. Thirty-seven (77.1%) were male. Asthma was the most common co-morbidity, affecting 5 (10.4%) of the 48 cases with an identified virus. The peak prevalence occurred during October (n=17). All patients required oxygen therapy and 17 (35.4%) required mechanical ventilation. The median length of hospitalization was 11 days. Preterm infants had a significantly higher rate of RSV infection than other respiratory viruses (8 of 21; 38% *vs* 3 of 27; 11.1%) (p=0.02). Viruses were most commonly associated with severe CAP among children aged less than 1 year. The peak prevalence occurred during the rainy season. Our findings suggest that young and preterm infants with CAP should be monitored closely due to their high risk for developing serious complications.

Keywords: viral infection, severe community-acquired pneumonia, children, Thailand

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INTRODUCTION

Pneumonia is a leading cause of hospitalization and death among children under 5 years (Shay *et al*, 1999; Williams *et al*, 2002; Henrickson *et al*, 2004). Common viral etiologic agents of pneumonia

in children are respiratory syncytial virus (RSV), parainfluenza (PIV), influenza, adenovirus, rhinovirus and coronavirus (McIntosh, 2002). Although these viruses usually cause mild infections in children, some may develop severe pneumonia, leading to respiratory distress and/or respiratory failure, necessitating mechanical ventilation and admission to a pediatric intensive care unit. Although these cases have a relatively high mortality rate they have not been well-studied in Thailand in this population group.

Respiratory viruses are commonly detected by viral cultures, direct immunofluorescent (DIF) assays, polymerase chain reaction (PCR) or real time PCR (RT-PCR) (Sazawal and Black, 2003; Rudan *et al*, 2008; Ginocchio *et al*, 2009). Viral cultures are considered the gold standard for detecting respiratory viruses, but this technique has limited availability and can only detect a small number of viruses. Viral culture yield depends on the quality of the sample, storage, transportation and the type of cells used (Ginocchio, 2007). Because the technique takes days to weeks to give results, it is not useful for patient management. DIF assays are more easily available and more rapid but less sensitive than cultures for detecting certain pathogens (Ginocchio, 2007). PCR and RT-PCR are both sensitive and rapid (Kehl *et al*, 2001; Gruteke *et al*, 2004; Jennings *et al*, 2004). PCR can identify multiple viruses. In our study we aimed to identify respiratory viruses among infants and young children with severe community-acquired pneumonia at a tertiary care hospital in eastern Thailand, using PCR and RT-PCR techiques.

MATERIALS AND METHODS

This study was conducted at Chonburi Hospital, a tertiary care hospital in eastern Thailand. It is a referral center for patients from nearby provincial hospitals.

Sample population

Samples were collected from infants and children aged 1 month to 5 years diagnosed with severe CAP and hospitalized at Chonburi Hospital between June 2013 and May 2014. Severe CAP was diagnosed following WHO criteria: fever ≥37.8°C, tachypnea for age (<2mo: >60/min, 2-12mo: >50/min, 1-5yrs: >40/min), cough or difficulty breathing with an oxygen saturation <90% or central cyanosis, severe respiratory distress (lower chest wall indrawing, grunting), abnormal lung auscultation (*eg*, coarse crackles, bronchial breath sounds) or signs of pneumonia with a general danger sign (inability to breastfeed or drink, lethargy or reduce level of consciousness, convulsions). Demographic data, clinical presentation, comorbidities, laboratory data, treatment and length of stay were recorded. Patients were excluded if no consent was obtained or if nasopharyngeal secretions were not available for analysis.

Laboratory testing

Samples were stored in viral transport medium at the site of collection and analyzed at the Center of Excellence in Clinical Virology within 72 hours of hospitalization. Extraction of viral nucleic acid was done using Viral Nucleic Acid Extraction Kits (RBC Bioscience, New Taipai City, Taiwan) following the manufacturer's instructions. Laboratory analysis involved screening by RT-PCR for influenza A and B viruses (Suwannakarn *et al*, 2008) and via an in-house PCR assay for human respiratory syncytial virus (RSV; Auksornkitti *et al*, 2011), rhinovirus (RV), Linsuwanon *et al*, 2009), adenovirus (AdV; Sriwanna *et al*, 2013), parainfluenza virus (PIV), Ruampunpong *et al*, 2014) and coronavirus (CoV).

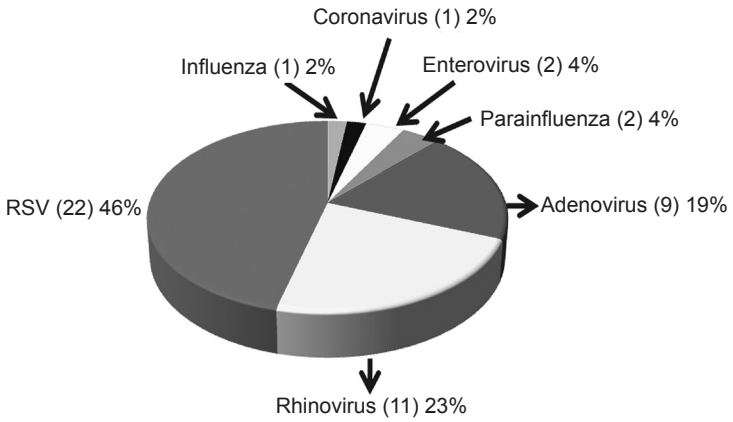

Fig 1–The distribution of viruses found among study subjects (*n*) %.

Statistical analysis

The data were presented as percentages, medians or means, ± SD. The ANOVA was used for multiple comparisons. The chi-square test was used for categorical analysis. All statistical analyzes were performed using SPSS software (version 20, IBM, Armonk, NY). A *p*-value <0.05 was considered statistically significant.

Ethical approval

This study was approved by the Institutional Review Board of the Chonburi Hospital, Ministry of Public Health, Thailand. Parents provided written consents on behalf of their children and were informed of the study objectives.

RESULTS

During the 1-year surveillance period, there were 257 cases of CAP diagnosed in children, of whom 102 (39.6%) had severe CAP. Nasopharyngeal swabs were obtained from 91 (89.2%) of the children with severe CAP. PCR was conducted in 48 cases (52.7%). Eleven cases were excluded from the study: 6 were sampled after 72 hour hospitalization and 5 were re-admissions.

Forty-eight cases had an identifiable respiratory virus. The most common viruses were RSV (*n*=22; 45.8%), RV (*n*=11; 22.9%) and Adv (*n*=9; 18.7%) (Fig 1). The month with the greatest number of cases was October (*n*=17).

Patient ages ranged from 1 month to 4 years 5 months, with a median age of 1 year 1 month. Thirty-seven patients (77.1%) were male. Sixteen patients (33.3%) infected with a respiratory virus also had at least one comorbidity. Asthma and allergic rhinitis were the most common comorbidities (8 of 48, 16.7%), followed by congenital heart disease (5 of 48, 10.4%). We also found a high prevalence of LBW (13 of 48, 27.1%), preterm births (10 of 48, 20.8%) and day care attendance (9 of 48, 18.8%). There were equal numbers with a history of passive smoking (*n*=9, 18.8%) and a family history of atopy (*n*=9, 18.8%) (Table 1).

Fever and cough were present in all patients. Rhinorrhea was found in 39 cases (72.2%), vomiting was seen in 13 (24%) and diarrhea in 8 (14.8%). Crepitations was the most common abnormal lung sign (66.7%). The mean oxygen saturation at presentation was 91% (60%-99%) (Table 2).

Laboratory findings

The mean leukocyte count was 12,080 (9,400-16,480 cell/mm^3). The mean percentage of neutrophils was at 44.7% (31%-59%) and the percentage of lymphocytes was at 38% (21%-49%). All patients studied had a chest radiograph prior to admission. The most common abnormality seen on chest

Table 1
Demographic data of patients enrolled in study.

Demographic data	Severe pneumonia (*N*=48) *n* (%)
Age (years)	
<1	31 (65)
1-5	17 (35)
Age, median (range)	1 year 1 month (1 month - 4 years 5 months)
Male gender	37 (77.1)
Underlying diseases	
Asthma / allergic rhinitis	8 (16.7)
Congenital heart disease	5 (10.4)
Bronchopulmonary dysplasia	2 (3.7)
Spastic cerebral palsy	2 (3.7)
Gastroesophageal reflux (GERD)	2 (3.7)
Patient characteristic	
Birth weight (<2,500 g)	13 (27.1)
Preterm birth	10 (20.8)
Passive smoking	9 (18.8)
Day care attendance	9 (18.8)
Family history of atopy	9 (18.8)

Table 2
Clinical presentation, laboratory and radiolgy results of study subjects.

	Severe pneumonia (*N*=48) *n* (%)
Clinical presentation	
Rhinorrhea	39 (72.2)
Vomiting	13 (24)
Diarrhea	8 (14.8)
Crepitations	36 (66.7)
Wheezing	16 (29.6)
Oxygen saturation on room air, mean (range)	91% (60-99)
Complete blood count	
WBC count (cell/mm^3)	12,080 (9,400-16,480)
Neutrophil count	44.7 (31-59)
Lymphocyte count	38 (21-49)
CXR	
Perihilar infiltration	29 (60.4)
Patchy infiltration	10 (20.8)
Atelectasis	9 (18.8)

Table 3
Comparison between patients with RSV infection and other viral infections.

	RSV (total = 21) *n* (%)	Non-RSV (total = 27) *n* (%)	*p*-value
Sex ratio (male : female)	2.5: 1	4.4: 1	0.32
Age, years			
≤1	15 (71.4)	16 (59.2)	0.54
1-5	6 (28.6)	11 (40.8)	
Patient characteristics			
Preterm	9 (42.8)	5 (18.5)	0.02*
LBW	8 (38)	3 (11)	0.04*
Exposure to second-hand smoke	3 (14)	6 (22.6)	0.39
Day care attandance	0 (0)	2 (7)	0.31
Family history of allergic disease	3 (14)	6 (22.2)	0.46
Temperature in °C, mean ± SD	37.8 ± 0.61	37.7 ± 0.94	0.91
Oxygen saturation on room air, mean (IQR)	95.7 (85-100)	93.0 (89-98)	0.06
Wheezing	4 (19)	11 (55.5)	0.10
CBC, cells/mm^3			
WBC, median (range)	12,930 (8,740-6,480)	15,085 (10,957-18,722)	0.22
Percent neutrophils (range)	44.4 (19.2-58)	50.5 (34.9-62.2)	0.43
Percent lymphocytes (range)	43.7 (34.9-60)	39.1 (23.3-50.6)	0.50
Treatment			
Duration of oxygen therapy in days, median (range)	3 (1-20)	2 (1-13)	0.66
Duration of mechanical ventilator therapy in days, median (range)	7 (3-10)	9 (2-19)	0.56
Antibiotic use, *n* (%)	13 (61.9)	18 (66.7)	0.55
Length of stay in days, median (range)	5 (2-35)	7 (2-73)	0.17

*Significant difference ($p<0.05$).

radiograph was a perihilar infiltrate (29 of 48, 60.4%). Patchy infiltrates were identified in 10 of 48 (20.8%) (Table 2).

Treatment

All patients in this study required oxygen therapy and 17 (35.4%) required mechanical ventilation.

Clinical outcomes

The median length of hospitalization was 11 days. The median duration of oxygen therapy was 5.2 days (1-20 days) and the median duration of mechanical ventilation was 6.8 days (2-21 days). The majority of subjects (87.5%) received inhaled bronchodilators and antibiotics (64.5%) despite the viral etiology. Respiratory failure occurred in 17 patients (35.4%). Three (6.3%) had concomitant bacterial pneumonia found by tracheal suction culture.

Comparing patients with RSV versus other respiratory viruses, we found LBW infants subjects born prematurely or with a LBW were more likely to have RSV infection (8 of 21; 38% *vs* 3 of 27; 11.1%) (p=0.02). Other risk factors, such as explosive to second-hand smoke, day care

attendance and family history of allergic disease were not significantly associated with type of viral infection (Table 3).

DISCUSSION

Severe viral community-acquired pneumonia in young children was common in our study, especially in those aged less than 1 year. Fever, cough, and rhinorrhea were among the most common clinical presentations of viral pneumonia in our study. The initial laboratory findings for white blood cell count, neutrophil count, and lymphocyte count could not distinguish between types of respiratory virus. The most common radiographic finding among subjects was a perihilar infiltration. This was common among study subjects but did not help distinguish among the viruses.

We detected respiratory viruses throughout the year with a peak during the rainy season. The most commonly found virus was RSV, the same as a previous study from Thailand performed during 2006-2007 (Fry *et al*, 2010). We detected a higher prevalence of RSV during August and October, during the rainy season, similar to a study from Thailand performed during 2003-2007 (Samransamruajkit *et al*, 2006) which found RSV was more common among children aged less than 5 years and the highest prevalence was from June to October.

Other subjects in our study were infected with influenza virus, parainfluenza virus, coronavirus and enterovirus. There was no correlation between clinical characteristics and seasonal outbreaks. Preterm and low birth weight infants had significantly higher rates of RSV infection in our study. These population groups tend to develop more serious complications (Law *et al*, 1998).

In conclusion, severe viral CAP occurred mostly in children aged less than 1 year in our study with a peak prevalence during the rainy season. RSV was the most common cause of severe viral CAP in our study followed by RV. RSV infection was significantly more likely to occur in LBW and premature infants. These infants need to be monitored closely if they develop CAP since they are at highest risk of developing serious complications.

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