ETIOLOGY OF ACUTE LOWER RESPIRATORY TRACT INFECTION IN CHILDREN AT SRINAGARIND HOSPITAL, KHON KAEN, THAILAND

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Abstract. We investigated the etiology of acute lower respiratory infection (ALRI) in children under 5 admitted to Srinagarind Hospital. The causative bacteria and viruses were determined by hemoculture and viral isolation from blood and nasopharyngeal aspirate samples. Antigens of respiratory syncytial virus (RSV) and Chlamydia trachomatis were detected using EIA. The 74 children less than 5 years of age with ALRI enrolled in our study were diagnosed with pneumonia (75.7%), croup (16.2%), and bronchiolitis (8.1%), respectively. Examination of blood or nasopharyngeal aspirate revealed viral or bacterial infections in 26 and 22 cases, respectively, whereas 5 of the children aged under 1 year (10%) were diagnosed with pneumonia caused by Chlamydia trachomatis. RSV was the most common virus detected (24.3%) and was associated with pneumonia and bronchiolitis, while the parainfluenza virus was the primary cause of croup. In cases of pneumonia, bacterial infections were identified in almost all of the cases: and Streptococcus pneumoniae and Haemophilus influenzae were the most commonly isolated (at 8.9% each). Mixed infections were detected in 8 cases (10.8%). The incidence of RSV infection peaked during the especially warm and cool seasons, whereas the bacterial infections were primarily associated with the relatively cool season. Our study indicates that a combined pneumococcal and Hib vaccine and a RSV vaccine would reduce the high rate of pneumonia in children under 5 years of age in Northeast Thailand.

INTRODUCTION

An acute lower respiratory tract infection (ALRI) is a leading cause of morbidity and mortality amongst children in developing countries (Denny and Loda, 1986). In these countries, it causes 19% of all deaths among children younger than 5 years and 8.2% of all disability (Shann et al, 1999). In developing countries, it is estimated that ALRI kills 4 million children every year: 3 million of these die from pneumonia (Shann et al, 1999). In Thailand, ALRI accounts for ~25% of deaths in children under one year. Of the children treated at general hospitals, 40 to 60% suffered from acute respiratory infection (ARI), and about 10% of them required hospitalization (Ministry of Public Health, 1980). In several studies, including a community-based study of ARI in Thailand, Streptococcus pneumoniae, Haemophilus influenzae, as well as the respiratory syncytial virus (RSV), the parainfluenza virus and adenovirus were the main bacterial and viral etiologies identified (Nohynek et al, 1991; Korppi et al, 1993; Torzillo et al, 1999; Vathanophas et al, 1990). In Northeast Thailand, many children live in low income families, with working mothers. Between 1973 and 1978, 300, 406 and 137 cases of ARI were reported in children aged under a year, between 1 and 5 years, and between 5 and 15 years, respectively. Nearly 5% of these 843 cases died from pneumonia, acute bronchiolitis, empyema and diphtheria. Despite its importance, there is no data on the etiology of ALRI in children living in Northeast Thailand.
which in turn limits the development of standard treatment protocols and the consideration of vaccines for prevention of ALRI.

Our report is a prospective study to determine the etiology of ALRI in children under 5 years of age admitted to Srinagarind Hospital, Khon Kaen, Thailand.

MATERIALS AND METHODS

Patients

We investigated patients under 5 years of age who were diagnosed with ALRI according to the pro forma developed for the WHO Global Program for Control of ARI (WHO, 1985; 1990) and admitted to the Department of Pediatrics, Srinagarind Hospital, Khon Kaen University between August 1992 and November 1994. After receiving verbal informed consent, a research nurse conducted a detailed interview for our clinical records comprising information on birth, past medical history, immunization status, history of present illness, feeding practices and family details. The study details and hospital records were reviewed after the discharge of each patient. Children over 5 or not fulfilling the WHO’s clinical and radiological criteria for ALRI were excluded.

Specimen collection and storage

Blood and nasopharyngeal aspirates (NPA) were collected from children on admission to hospital. NPA were collected by a catheter to the nasopharynx and rinsed into a test tube with 3 ml of viral transport media (Hank’s balanced salt solution supplemented with 0.4% bovine serum albumin) and immediately transported on ice to the virology laboratory. NPA specimens were processed for respiratory viruses and Chlamydia trachomatis. The remaining portions of the NPA specimens were stored at -70°C.

Bacterial culture

Blood was cultured for bacteria up to 7 days and was subcultured as needed on days 1, 3 and 7.

Viral studies

NPA was centrifuged at 2,000 rpm for 10 minutes, and 0.2 ml of the supernatant was inoculated into duplicate tubes of cell culture as follows: Vero, HEp-2, MDCK, LLC-MK, and MRC-5. Culture was incubated in MEM media containing 2% fetal calf-serum (FCS) and with penicillin (200 units/ml), streptomycin (200 µg/ml), kanamycin (100 µg/ml) and fungizone (1 µg/ml), respectively. The cultures were incubated at 37°C for up to 3 weeks and examined every 1 to 2 days for detection of cytopathic effects. Cultures with cytopathic effects or with positive hemadsorption were further tested by indirect immunofluorescence for positive identification.

Immunofluorescence test (IF)

After the NPA pellet was washed three times with PBS by centrifugation, the NPA deposit was re-suspended in a small quantity of PBS, and spotted onto multi-well slides. The slides were air-dried, fixed in acetone for 10 minutes and stored at -70°C. The slides prepared from virus cultures showing cytopathic effects or hemadsorption were also prepared as above. All the slides were processed with antisera from Welcome Diagnostics (Dortford, England) specific for influenza viruses A and B, para-influenza viruses types 1, 2 and 3, RSV, adenovirus and herpes simplex virus (HSV), and examined under fluorescence microscope.

Enzyme immunoassay (EIA)

RSV antigen detection: Dakopatts avidin-biotin amplified ELISA system was used for direct antigen detection of RSV in NPA secretion (Varsano et al., 1995). Nunc 96-well immunoplates were coated for 2 hours at room temperature (RT) with rabbit anti-RSV and, in the control, with rabbit normal immunoglobulin fraction diluted in a coating buffer at pH 7.2. Plates were washed between steps with a phosphate buffer containing 0.1% Tween 20 (PBS-Tween). The NPA samples were sonicated and 1:2 dilution samples inoculated into the coated wells where they were incubated for
1 hour at 37°C. After washing, biotinated anti-RSV serum was added to the well and incubated for 30 minutes at RT. Next, peroxidase conjugated avidine was added and incubated for another 30 minutes. After washing, substrates were put into the wells, and incubated for 15 minutes at RT, then the reaction was stopped with 0.1 ml of 1 M H₂SO₄ per well. The results were read in an ELISA reader at 492 nm.

**Chlamydia antigen detection:** Enzygnost chlamydia (Behring) is an EIA kit for the detection of the chlamydia antigen in NPA secretions. Assay procedures were done using a standard protocol. The NPA secretion was vortexed, boiled for 10 minutes and re-vortexed. An aliquot of the specimen and control were dispensed into a micro-well along with a murine IgG monoclonal antibody specific for lipopolysaccharide of chlamydia and horse-radish peroxidase conjugated anti-murine IgG antibody. Following incubation, the wells were rinsed and a chromogenic substrate, 3′3′,5′-tetramethyl benzidine (TMB), was added. The reaction was stopped by adding 50 µl of 1 M H₂SO₄ and plates were read at 450 nm with an ELISA reader (Hammerschlag et al, 1990).

**RESULTS**

Between August 1992 and November 1994, 74 patients, under 5 years of age, diagnosed as ALRI, were admitted to the Department of Pediatrics at Srinagarind Hospital, Khon Kaen University, and enrolled in our study. Forty were male. Patients were predominantly (67.6%) under 1 year of age. The most common diagnosis was pneumonia (75.7%), followed by croup (16.2%) and bronchiolitis (8.1%), respectively. Pneumonia presented more commonly in children under a year (Table 1). The etiologic organisms found in these cases were identified (Table 2). Bacteriology

Blood cultures were taken and bacteria were identified in all cases of pneumonia (56 cases) and were positive in 22 cases (39.3%) (Table 3). The identification of the bacterial isolates is shown in Table 2. *Streptococcus pneumoniae* and *Haemophilus influenzae* were the most common bacteria found (each with 6.8%) (Table 4). Of the 50 ALRI cases in children aged under a year, 5 (10%) were chlamydia infection. The etiologic organisms found in these cases were identified (Table 2).
positive for chlamydia, and all of these developed pneumonia (Table 3).

**Viral studies**

All of the NPA from the 74 cases were examined by viral culture, immunofluorescence and EIA (RSV antigen detection). Evidences of viral infection were detected in all of the diseases identified (Table 2). RSV was the most common (24.3%) accounting for 75% of all viral isolates. Most of the RSV infections were associated with pneumonia and bronchiolitis, while the parainfluenza virus types 1 and 3 were associated with croup (Table 4).

**Coinfection**

Coinfection was also confirmed in 8 cases (10.8%). Of the 22 cases with bacterial infection, 7 had a mixed bacterial infection, while 1 of the 26 cases of viral etiology had a mix of two kinds of viruses.

Regarding the incidence of infection, bacteria and viruses were detected in all age groups studied except in those aged 4 to 5 years. Most of the children in our study were under a year old, and they had the lowest positive detection rate (Table 5).

To evaluate the seasonal trend of ALRI, the seasonal variations were observed in cases of viral infection, particularly RSV. The RSV detection was associated with the high temperatures (36°C) and relative humidity common between June and August and with low temperatures (15-20°C) common between Sep-
DISCUSSION

Our study was conducted between August 1992 and November 1994, and was the first study to explore the etiology of ALRI in children under 5 years of age admitted to Srinagarind Hospital, Khon Kaen, Thailand. Results of our study corresponded with the prevalence of ALRI in developing countries, in which pneumonia was the most common diagnosis, followed by bronchiolitis and croup (Chan et al., 1999; Vuori et al., 1998). Age- and gender-specific incidences of ALRI in our study were comparable to those observed in other countries (ie, children aged under a year being the most affected and boys more than girls) (Vathanophas et al., 1990; AHRTAG, 1986) were the bacterial and viral agents (Torzillo et al., 1999; Zhang et al., 1986). Our study provided further evidence that a large proportion of ALRI is associated with *Streptococcus pneumoniae*, which is the leading causative agent of pneumonia and detected in both ambulatory and hospitalized patients with equal frequency (Korppi et al., 1993; Vuori et al., 1998; Heiskanen-Kosma et al., 1998).

Like studies from other developing countries (Vathanophas et al., 1990; Berman, 1991), our study found a similarly high frequency of *Haemophilus influenzae* and *Streptococcus pneumoniae* in blood cultures. By contrast, *Haemophilus influenzae* was rarely reported in children with ALRI from developed countries (Torzillo et al., 1999; Vuori et al., 1998) perhaps mitigated by vaccination programs. Therefore, Hib and *Streptococcus pneumoniae* vaccines and standardized antibiotic treatments should be introduced for children in developing countries.

*Chlamydia trachomatis* was detected in 5 of 50 of the cases of ALRI in children under 1 (10%). Most of them were identified as pneumonia. Conflicting findings were reported in a study of 90 hospitalized children in the Gambia (Harrison et al., 1978), in which only 0.22% of infants had evidence of a *Chlamydia trachomatis* infection. However, 19.2% of 255 Argentinean children aged 1 to 18 months, had evidence of *Chlamydia trachomatis* infections associated with ALRI (Carballal et al., 1992).

In the United States, 30 to 35% of pneumonia in the first four months of life is attributed to *Chlamydia trachomatis* (Harrison et al., 1978; Schachter, 1978). Despite a difference in the rate of infection, previous studies and ours found chlamydia played a significant role in pediatric pneumonia, especially in children under a year old.

Data on the viral etiology of ALRI showed the detection rate varies between 22 to 55% depending on method used (Torzillo et al., 1999; Chan et al., 1999; Tecu et al., 1996; Yilmaz et al., 1999). RSV was the most frequently encountered agent. In our study, 35.1% of ALRI cases were viral positive and RSV was the most commonly detected, especially among those suffering from pneumonia and bronchiolitis.

<table>
<thead>
<tr>
<th>Agents</th>
<th>0-1 year (n = 50)</th>
<th>1-2 years (n = 8)</th>
<th>2-3 years (n = 8)</th>
<th>3-4 years (n = 7)</th>
<th>4-5 years (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>16 (32.0)</td>
<td>5 (71.3)</td>
<td>2 (25.0)</td>
<td>3 (42.9)</td>
<td>0</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>5 (10.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacteria</td>
<td>11 (22.0)</td>
<td>2 (25.0)</td>
<td>6 (75.0)</td>
<td>3 (42.9)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>32 (64.0)</td>
<td>7 (87.5)</td>
<td>8 (100)</td>
<td>6 (85.7)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5

Relationship between the etiologic agent group and the age group of children with ALRI.
Our results demonstrate that the etiology of ALRI in children is a complex issue. The high proportion (10.2%) of multiple infections might make it difficult to control ALRI. Since either the first-line antibiotic treatment for either \textit{Streptococcus pneumoniae} or \textit{Haemophilus influenzae}, and the biochemical or radiologic predictors of a particular organism are unlikely to be helpful, an optimal methodological protocol for studying the etiology of ALRI should therefore be considered.

Although, our study showed a large number of positive cases of blood culture (39.3%), the low culture yield coincided with earlier reports (Vuori \textit{et al}, 1998; McCarthy \textit{et al}, 1982). Some researchers have reported a high incidence of detection rate by using a combination of methods (Vuori \textit{et al}, 1998; Hijazi \textit{et al}, 1996). Our study also confirmed that the RSV detection rate increased (7 to 18 cases), when EIA was used as a supplementary method. The problem of finding an etiologic diagnosis of ALRI is due to the lack of a reliable gold standard detection method.

In conclusion, our data provide the prevalence and etiology of ALRI in the children under 5 years of age. Pneumococcal as well as Hib vaccines would significantly reduce the high rate of pneumonia, despite the high rate of co-infections and unsuspected microorganisms. Additionally, a better and more rapid diagnostic tool is needed to ensure an appropriate treatment is given.

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