Journal of Clinical Virology 56 (2013) 7–12

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Clinical features of radiologically confirmed pneumonia due to adenovirus in children

Shih-Perng Chen[a,d], Yhu-Chering Huang[a,b,*,d], Cheng-Hsun Chiu[a,b], Kin-Sun Wong[a,b], Ya-Ling Huang[b,c], Chung-Guei Huang[b,c], Kuo-Chien Tsao[b,c], Tzou-Yien Lin[a,b]

[a] Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[b] Chang Gung University College of Medicine, Taoyuan, Taiwan
[c] Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 3 August 2012
Accepted 27 August 2012

Keywords:
Adenovirus
Children
Pneumonia

ABSTRACT

Background: Clinical features of radiologically confirmed pneumonia due to adenovirus in children have not been comprehensively evaluated.
Objective: To describe the detailed clinical features of radiologically confirmed adenovirus-associated pneumonia in children.
Study design: Of 3298 children with a throat virus culture positive for adenovirus treated at a university-affiliated hospital, from January 2000 to June 2008, in northern Taiwan, 80 hospitalized children (2.4%) with radiologically confirmed pneumonia were identified. From four children with incomplete medical records, only demographics were included for analysis.
Results: The median age was 2.97 years, ranging from 25 days to 14 years. Seventy-three patients (96%) had fever, with a median duration of 7 days. The three most common respiratory symptoms were cough (99%), rhinorrhea (82%) and dyspnea (42%). Gastrointestinal symptoms were recorded in 80% of the patients, and neurologic symptoms in four children. Leukocytosis (WBC $\geq$ 15,000/μL) was noted in 19 (25%) patients. Only six patients (8%) had a normal serum C-reactive protein (CRP) value (<5 mg/L), while 48 patients (63%) had a CRP level >40 mg/L. Seventeen (21%) children required intensive care. Seventy-three patients (96%) recovered uneventfully. Sequelae were seen in two patients and death in 1. Of the 69 isolates with serotyping determination, seven serotypes were identified, with a predominant serotype (type 3 for 73%).
Conclusion: Less than 5% of the children with adenoviral infection had radiologically confirmed patch pneumonia. The manifestation of pneumonia caused by adenovirus was similar to that of bacterial pneumonia.

1. Background

Adenovirus, a double-stranded DNA virus, is one of the major pathogens of acute respiratory illness in children. [1] Adenoviruses are most frequently associated with upper respiratory tract syndromes such as pharyngitis or coryza, but can also cause lower respiratory tract infection including bronchiolitis, croup and pneumonia.[1–4] Less frequently, adenovirus can cause other manifestations such as gastrointestinal, ophthalmologic, genitourinary and neurologic diseases.[1,5–15] Adenovirus infections can occur sporadically or in epidemics and can be identified throughout the year.[16] At least 51 serotypes were identified and different serotypes may be implicated in different disease spectrum.[1] For example, nasopharyngitis and tonsillitis are commonly associated with serotypes 1, 2, 3, 5 and 7; pharyngoconjunctival fever is usually caused by serotypes 2, 3, 4 and 7; pneumonia is usually caused by serotypes 3, 7 and 21; acute gastroenteritis is commonly caused by serotypes 40 and 41; and acute hemorrhagic cystitis is commonly caused by serotypes 7, 11 and 21.[1]

Pneumonia caused by adenovirus varies in severities, ranging from bronchopneumonia to life-threatening acute respiratory distress syndrome (ARDS), and even fatal ones.[2,17–21] Acute adenoviral pneumonia is difficult to be distinguished from bacterial infections[18–23] and may also lead to long-term respiratory sequelae.[18,24] There have been scanty reports regarding detailed

* Corresponding author at: Division of Pediatric Infectious Diseases, Chang Gung Memorial Hospital, No. 5, Fu-Shin Street, Kweishan, Taoyuan, Taiwan.
Tel.: +886 3 3281200x8202; fax: +886 3 3288597.
E-mail address: ychuang@adm.cgmh.org.tw (Y.-C. Huang).
[d] These authors equally contributed to this manuscript.

1386-6532/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2012.08.021

description of epidemiology and clinical manifestations of radiologically confirmed pneumonia due to adenovirus in children.

2. Objectives

To better understand the epidemiology, clinical manifestations and outcomes of radiologically confirmed adenoviral pneumonia in children.

3. Study design

3.1. Case enrollment

A total of 3298 patients with a throat virus culture positive for adenovirus were treated at Chang Gung Children's Hospital and Chang Gung Memorial Hospital from January 2000 to June 2008. Among these patients, less than 5% were out-patient cases. All medical records as well as chest radiologic films, if taken, were retrospectively reviewed. Eighty hospitalized children (2.4%) less than 18 years of age were found to have pneumonia, which was defined as at least a patchy density on the chest radiologic film with compatible clinical symptoms/signs, and were included in this study. The patients, according to intensive care unit (ICU) admission or not, were categorized into two groups, namely, ICU group and non-ICU group.

Of the 80 patients, four children had unavailable or incomplete medical charts (including three patients in the ICU group) for detailed review, and from them, only epidemiologic and demographic data were extracted for analysis. A total of 76 hospitalized patients were finally analyzed for clinical presentations, laboratory findings, and outcomes.

3.2. Definitions

Pneumonia was defined as at least a patchy density on chest X-ray films with clinical respiratory manifestations. Fever was defined as body temperature $\geq$38 °C; high fever was body temperature $\geq$39 °C. Leukocytosis was referred to white blood cell (WBC) count $\geq$15,000/μL. Bacterial co-infection was defined as evidence of positive bacterial cultures from sterile sites. If a patient had a positive urine test for *Streptococcus pneumoniae* antigen but without a positive culture from the sterile site, the case was categorized as probable pneumococcal co-infection. Patients were considered to be co-infected with *Mycoplasma pneumoniae* if a positive serologic test for specific IgM was documented. Concomitant viral infection was defined if the viral culture identified another viral pathogen along with adenovirus. When other organ system than respiratory system was involved, the disease spectrum was categorized as disseminated disease. Hepatic involvement was defined as elevated serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) level twice the upper limit of normal value.

3.3. Virology

Specimens were obtained from throat swabs, collected and transported in a viral transport media to the virologic laboratory within 4 h. Viral isolation was carried out by tissue cultures. Respiratory specimen was inoculated into four different cell lines, including human epidermoid carcinoma cell (Hep-2), canine kidney cell (MDCK), human embroyonal lung cell (MRC-5), and rhesus monkey kidney cell (MK-2). Cultures were maintained in minimal essential media containing antibiotics and incubated at 33 °C, rotated at 12 revolutions/h. All cultures were observed daily for cytopathic effects and identified using adenovirus specific monoclonal antibody (Chemicon, CA) by immunofluoresent assay.[18,22]

3.4. Genotyping

Of the 76 patients, 70 viral isolates were available for genotyping by sequence-based method. Aliquots 200ul of each of isolates known to be adenovirus culture positive from the clinical virology laboratory were retrieved from −80 °C storage. Briefly, viral DNA was extracted from 200 μL of the adenovirus-positive viral isolates in a final elution volume of 60 μL using the Labturbo Virus Mini Kit (Taigen Bioscience Corp., Taipei, Taiwan). An initial volume of 5 μL of eluted sample was amplified using a PCR procedure that targeted hyper variable regions 1–6 of the hexon gene. M13 universal priming tails were added to the primers to facilitate sequencing.[25] Expected amplicons from this reaction ranged from 764 bp to 896 bp in length. These amplicons were then sequenced on the ABI PRISM® 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The forward and reverse sequences were generated from the assembly using the programs SeqMan in the Lasegene software package (DNASTAR, Madison, WI, USA) .The database was available in GenBank using a BLAST. The serotype of each virus isolate to be identified was determined by the serotype of the highest scoring strain in the database, which is the sequence of the adenovirus strain that gave the highest nucleotide similarity value with the query sequence.

3.5. Statistics

Data were analyzed by the SPSS program. Student *t* test was used for continuous variables and Chi square test were used for categorical data. A *P* value less than 0.05 was denoted to be statistically significant.

4. Results

During the study period, adenovirus was identified all year-round but the number of isolates was higher during winter seasons. Among the 80 cases of adenoviral pneumonia, the epidemiological distribution was even throughout the year. There were 9–10 cases per month during the spring season of March–May; same case number per month was also seen in October and January. Only four to five cases per month were identified in the rest of months (Fig. 1).

Of the 80 children, the median age was 2.97 years, ranging from 25 days to 14 years. Thirty percent of the patients were less than 2 years of age and more than 90% (93%) were less than 6 years of age. There were 41 males with a male-to-female ratio of 1.05. Seventeen (21%) children were ever admitted to ICU. The median length of hospital stay was 7 days; the range was from 3 days to 65 days. Duration of hospitalization was significantly longer for the children in ICU group than in non-ICU group ($P < 0.001$). The detailed demographic data are shown in Table 1.

Table 2 illustrates the detailed clinical symptoms of these 76 children. Almost all patients (96%) had fever, and 75% of the patients had fever greater then 39 °C. The median peak body temperature was 39.2 °C (range, 37–41 °C). The median duration of fever was 7 days, ranging from 0 day to 16 days. The three most common respiratory symptoms were cough (99%), rhinorrhea (82%), and dyspnea (42%). Extra-pulmonary manifestations were also documented. Gastrointestinal symptoms were reported in 80% of the patients, including vomiting in 24 patients, diarrhea in 25 patients and abdominal pain in 12 patients. Six patients (7.8%) had ileus. Neurological symptoms were seen in 4 patients (5.2%); all of them had seizure, with febrile seizure in 3 and encephalitis in 1. Five patients had hepatic involvement. Dyspnea was significantly frequently seen in patients of the ICU group than those of non-ICU group. Acute respiratory distress syndrome (ARDS) was diagnosed in one patient who subsequently died. Fifteen (20%) were