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Hospital-acquired adenovirus 7h infantile respiratory infection in Chile

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Background. **Adenoviruses are the second most common cause of viral acute lower respiratory tract infection (ALRI) requiring hospitalization in Chile. Little information is available with respect to nosocomial infection rate by adenovirus. This issue is important because of its potential severity and long term sequelae.**

Methods. **Infants hospitalized for ALRI were studied to determine the rate of nosocomial cross-infection with respiratory adenovirus and its corresponding genome type. The group studied included all cases younger than 2 years of age admitted to a seven crib ward in the Roberto del Río Children's Hospital (Santiago, Chile) between May, 1995, and October, 1996. Nasopharyngeal aspirates for immunofluorescence assay and viral isolation were obtained on admission and the next day. On identification of a positive case for adenovirus, samples were obtained from contacts for 2 consecutive days and twice weekly thereafter for 2 weeks.**

Results. **Fifteen index positive cases for adenovirus and their 65 contacts were identified. Secondary attack rate for adenoviral cross-infection was 55%, most of which were diagnosed by viral isolation. Mortality occurred in 4 cases; 3 had underlying diseases. Four secondary cases presented mild respiratory infection after acquiring the cross-infection, and 16 patients developed a moderate and severe ALRI. Twelve patients required supplemental oxygen and 4 needed mechanical respiratory support. Genome types for the 10 index cases and 19 contacts were obtained. All of these corresponded to adenovirus 7h.**

Conclusions. **The high secondary attack rate observed, stresses the importance of adequate isolation of patients and the need for rapid and sensitive viral diagnosis.**

INTRODUCTION

Worldwide, acute lower respiratory tract infections (ALRI) are the main cause for hospitalization in infants, particularly in the cold season. They are the first cause of death in children between 1 month and 4 years of age.[1–3] In Chile respiratory viruses are the leading cause of ALRI in children <2 years of age.[4, 5] Epidemiologic studies of ALRI requiring hospitalization in Chile have detected respiratory syncytial virus (RSV) in 50 to 60% of the cases and adenoviruses in 12.6%. Adenoviruses are isolated all year long, whereas RSV is detected mainly during the cold season.[4] Adenovirus may cause severe pneumonia and wheezing bronchitis associated with prolonged hospital stay, admission to an intensive care unit, need for respiratory support and death.[6] There are still many hospitals in developing countries with large pediatric wards that contain many cribs and where patients are managed by the same staff nurses and doctors. The presence of an adenovirus case in a pediatric ward can cause hospital outbreaks with severe consequences to those infected.[7–10.] Additionally it results in an administrative burden associated with the required isolation of index cases, leading to ward closure to new admissions. Children with respiratory adenovirus may have a wide variety of presentations, from a flu-like syndrome to fatal pneumonia.[6] There is limited information about adenovirus nosocomial infection. In one report from a neonatal unit, there was a nosocomial infection rate of 30% for adenoviruses 2 and 3 in bronchopulmonary dysplasia patients.[8] There is no information with respect to nosocomial infection rates by respiratory adenovirus in Chile. This is a very important issue because of its potential severity and long term sequelae.[7, 10]

Adenovirus 3, 7 and 21 are associated with acute ALRI.[10] In Chile we isolate mainly serotypes 7, 2 and 1 from hospitalized infants.[11] The implementation of genome typing techniques using restriction enzymes has permitted definition of clinical and epidemiologic features associated with specific adenovirus strains.[12] Epidemiologic studies in Chile and in the southern cone of South America have demonstrated the emergence in 1984 of a genomic variant with greater patho-

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logic potential, adenovirus 7h.[13–15] In our data from 1995 genome type 7h accounted for 56% of 101 adenovirus isolates, 36% of them were community-acquired and 64% were hospital-acquired infections. Adenovirus 7h variant has been associated with longer hospital stays, pulmonary sequelae and death.[11, 12]

The aim of this study was to determine the nosocomial infection rate for respiratory adenovirus infections and to observe short and long term outcome of the secondary cases.

PATIENTS AND METHODS

Patients. We have performed epidemiologic surveillance of ALRI in children <2 years of age admitted to a nonisolated seven crib ward at the Roberto del Río Children's Hospital in Santiago, Chile, since May, 1988. Exclusion criteria included children with a history of prematurity, underlying pulmonary, cardiac or neurologic diseases and those who required previous hospitalization for any reason. Adenovirus infection cases were children <2 years of age admitted to the Roberto del Río Children's Hospital for ALRI, between May, 1995, and October, 1996. Clinical criteria for the diagnosis of ALRI included fever, tachypnea, wheezing, cough, dyspnea, rales, cyanosis, respiratory distress, abnormalities in chest radiographs and hypoxemia. Two samples of nasopharyngeal aspirates were obtained within 72 h of admission and processed for immunofluorescence assay and viral isolation in cell culture.

Ward description. At the Roberto del Río Children's Hospital, infants with ALRI were admitted to a seven crib ward that had ~1 m separation between cribs. They were cared for by the same staff nurse, who was in charge of feeding the children, nebulizing and changing diapers and clothes. A senior nurse was in charge of giving medicine to all patients in the same unit including four other wards. Patients were also in direct contact with medical staff, residents, interns and medical students. Each ward used the same sink. Parents were not allowed to stay with their children. Doctors, nurses and medical students frequently examined patients without using gloves and masks.

Contacts follow-up. On detection of a positive case for adenovirus (index case) by immunofluorescence assay, all the children admitted to the same ward at the same time had nasopharyngeal aspirates taken on 2 consecutive days and twice weekly thereafter for up to 14 days after the exposure. This was also done after hospital discharge through outpatient interviews. For each case a registry including diagnosis, name, age, date and time of admission and discharge and days and hours of exposure to adenovirus-positive cases was created. Results of viral isolation took ~7 days, on occasion delaying isolation of cases from the rest of the infants in the ward. Contacts were followed and a sample of nasopharyngeal aspirate for immunofluorescence assay and viral isolation was obtained every other day. If the children appeared symptomatic they were evaluated clinically in the hospital and outpatient clinic, with clinical findings being registered. According to the severity of the acute adenovirus respiratory nosocomial infection, patients were classified into four groups: mild (Group I), febrile illness, bronchitis or bronchiolitis; moderate (Group II), children requiring supplemental oxygen; severe (Group III), infants requiring intensive care; and respiratory assistance and fatal (Group IV).

Risk of cross-infection. For measurement of the intensity of exposure to adenovirus infection from an index case, the number of days of residence in the same ward was registered for each contact case. An arbitrary minimum of 48 h of exposure was required to enroll the case in the follow-up system.

Viral study. A nasopharyngeal aspirate was routinely obtained for each patient within the first 48 h after admission and a second specimen was obtained 24 h later. Indirect immunofluorescence assay and viral isolation in HEp-2 and MDCK cell lines for RSV, influenza virus, parainfluenza and adenovirus were conducted. Nasopharyngeal aspirates samples were collected by gentle suction through a plastic catheter with a specimen trap. Approximately 4 ml of viral transport medium (containing 0.5% gelatin and antibiotics) were suctioned through the catheter, thereby washing any of the specimen remaining in the catheter into the trap, and transported on wet ice to the virologic laboratory within 1 h.[4] The sample was centrifuged at 2000 rpm for 20 min, and the supernatant was inoculated into HEp-2 or MDCK cell culture. Cultures were incubated at 37°C and observed every other day for development of cytopathic effect for 1 week, after which confirmatory immunofluorescence assay for adenovirus was performed in both cultures, with and without cytopathic effect. For immunofluorescence assay the sample was processed as described elsewhere[4] using monoclonal or polyclonal anti-adenovirus antibodies provided by Drs L. Anderson (CDC, Atlanta, GA) and G. Wadell (Sweden), respectively, and with commercially available conjugates (SIGMA).[4] Genome typing of isolates from index cases and positive contacts were studied with restriction enzymes. Each strain was propagated in HEp-2 or A-549 cells; viral DNA was extracted according to the method of Shinagawa et al. and further studied with different endonucleases.[12–15] Isolates belonging to subgenus B were typed by digestion with the endonucleases *Bam*HI and *Sma*I and, for further characterization, *Xho*I. For subgenus C isolates the endonucleases used were *Bam*HI, *Sma*I, *Bgl*II and *Hin*dIII. Electrophoresis was run for 16 h at 50 to 60 V. The bands obtained on

1.2% agarose gels were visualized by staining with ethidium bromide, inspected under ultraviolet light and photographed. The electrophoretic patterns obtained were compared with international reference patterns to assign serotypes and genotypes to the strains analyzed.[12–15]

Long term follow-up. Thirty-three patients were followed for at least 2 years in the respiratory outpatient clinic by one of the authors (MAP). Patients were examined on a regular basis for clinical symptoms and sequelae, with chest roentgenogram, ventilation-perfusion scan and computer tomography performed when necessary. Hospitalizations and treatments were recorded as well. Patients who did not attend the clinic were contacted by telephone and asked to come for examination.

RESULTS

Follow-up of 65 contacts of the 15 index cases was obtained. Thirty-three patients were followed for 2 weeks from contact (51%) and the rest were followed for at least 1 week.

No difference in the average exposure time for contacts from index cases was observed in both cross-infected and noninfected patients. The mean time of contact with the index case was 2.7 days (range, 2 to 16 days). The median was 2 days.

Secondary adenovirus infection was detected in 36 cases for a secondary attack rate of 55%. Diagnosis for nosocomial infection was obtained by viral isolation alone in 88%.

Four secondary cases presented with febrile upper respiratory infection or mild lower respiratory infection (bronchitis or wheezing bronchitis) after acquiring the cross-infection and 16 patients developed a moderate or severe ALRI; 12 patients required supplemental oxygen and 4 received mechanical ventilator support. Five were readmitted whereas the others were still in hospital at onset of clinical symptoms resulting from adenovirus nosocomial infection. Four secondary cases died, 3 with underlying disease (bronchopulmonary dysplasia, Down's syndrome and congenital heart disease-associated pulmonary hyperemia). The case fatality rate for secondary adenovirus infection was 11%.

Ten index cases were genome-typed, all being adenovirus 7h. Genome type was also obtained from 19 contacts and all were adenovirus 7h. In only 2 of the 15 outbreaks did we fail to detect adenovirus 7h or other strains in either the index or secondary cases. No other adenovirus genotypes were identified. Secondary attack rates for each index case are presented in Table 1, which emphasizes the infectiousness of strain 7h.

Long term follow-up of 33 nosocomial adenovirus infection cases has revealed persistent pulmonary symptoms. Atelectasis was present in 7 cases and was the main sequelae observed after nosocomial adenovirus pneumonia. Chronic pulmonary disease (obliterative bronchiolitis, chronic atelectasis and bronchiectasis) was found in 2 cases, and bronchiectasis plus atelectasis was found in 1 patient.

DISCUSSION

Adenoviral infections produce a wide range of diseases, including upper respiratory infections, febrile flu-like syndrome, pharyngoconjunctival fever, severe pneumonia, enteric disease and hepatic disease. This far 49 adenovirus serotypes have been described, with variable virulence.[10] Our studies in Chile have demonstrated that serotypes 7, 2 and 1 are the most frequently detected in children hospitalized for acute ALRI, with genome type b7h accounting for ~50% of the cases.[11] In South America genome type 7h has been associated with pneumonia of increased severity, longer hospital stay and high mortality when compared

TABLE 1. Adenovirus strain genome types and secondary attack rate per index case: Roberto del Rio Children's Hospital, May, 1995 to October, 1996

Index Case	Index Case Genome Type	Contact's Genome Type	No. of Positive Contacts	No. of Negative Contacts	Secondary Attack Rate (%)
1	ND	7h (4)	4	2	67
2	7h	7h (2)	4	0	100
3	ND		3	1	75
4	ND		3	3	50
5	7h		3	0	100
6	7h	7h (2)	3	3	50
7	7h	7h (1)	1	2	33
8	7h	7h (1)	1	4	20
9	ND	7h (3)	3	3	50
10	ND	7h (1)	2	1	67
11	7h	7h (1)	2	2	50
12	7h		0	2	0
13	7h	7h (1)	2	0	100
14	7h		1	4	20
15	7h	7h (3)	4	2	67
Total			36	29	65

* Numbers in parentheses, number of typified strains.
ND, not determined.

with adenovirus pneumonia of other genome types.[11–14] Although immunofluorescence assay for adenovirus diagnosis can be easily set up in any clinical laboratory, its low sensitivity places it in clear disadvantage to isolation of virus in cell culture.[16] However, in our experience, immunofluorescence assay has been a sensitive diagnostic tool, allowing detection of adenovirus in 25% of the mild cases and in 60% of the severe infections. This increased sensitivity to diagnose severe adenoviral infections may be explained because a more aggressive strain could cause significant cell destruction, more intense cell replication and therefore larger and more prolonged viral shedding. The massive viral excretion may promote nosocomial infections, unless isolation of contagious patients and other control procedures are implemented. In developing countries hospitalizations take place in multiple crib wards and patient isolation is difficult. Early etiologic diagnosis to identify potential adenovirus carriers is essential to avoid secondary cases. Hospitalized children are frequently moved between wards and are followed up in different outpatient clinics on discharge, making secondary attack rates difficult to study. Not withstanding the above the analyzed experience permits an estimation of the problem of nosocomial infection by adenovirus in Chile. The majority of secondary cases were diagnosed within 2 weeks based on viral isolation alone.

Because of increasing survival rates in children with underlying chronic disease (pulmonary damage, congenital heart diseases, etc.), hospitalized children in the future will include an increasing number of these cases that are particularly high risk for severe adenovirus infection. In Chile the mortality rate from adenovirus pneumonia has been shown to be higher in children with underlying disease. Their condition increases their susceptibility to hospital-acquired infections because of longer hospital stays. The mortality rate of our study group was high, with a majority of cases with underlying diseases. Nosocomial infection is more severe than community-acquired infection. This possibly is because of a high infective dose. Another explanation could be that a more aggressive and contagious strain, such as adenovirus b7h, accounted for hospital-acquired infections. Our epidemiologic situation at the time of this study did not permit a comparison with other adenovirus strains, because all strains isolated were adenovirus 7h.

Adequate isolation of patients admitted for a respiratory condition suggestive of adenovirus must be emphasized. On the basis of this experience we propose the following scheme for hospitals with multiple crib wards: children with ALRI are admitted to an isolation ward and nasopharyngeal aspirates are taken. If RSV is present (RSV season) and there is no clinical suspicious of adenovirus infection, such as high fever and progressive pulmonary involvement, the patient is transferred to a multiple crib RSV ward. If immunofluorescence assay for adenovirus on admission is negative but the clinical symptoms are suggestive of adenovirus respiratory infection, serial immunofluorescence assay should be done.[11] Undoubtedly a rapid and more sensitive diagnosis is required to avoid the high secondary attack rate observed. In this respect rapid viral isolation techniques in shell-vials and PCR to detect viral DNA have been developed. Although less sensitive than classical isolation, they are more sensitive than immunofluorescence assay and should be considered in clinical management to limit the spread of nosocomial adenovirus.

The long term follow-up showed sequelae, like atelectasis, bronchiectasis and obliterative bronchiolitis. Frequently children with chronic lung disease attending pulmonary outpatient clinics do not have a history of bronchopulmonary dysplasia. They could have nosocomial adenovirus infections and not be recognized because they were discharged during the long incubation period, without further study. Symptoms could have appeared after discharge and the children might have been seen in another institution, where a nosocomial infection was not considered because of lack of proper information.

This is the first study in Chile measuring the risk of acquiring nosocomial adenovirus respiratory infection. The high secondary attack rate observed in this study, in addition to potential seriousness of adenovirus infection, should promote more detailed studies to determine factors influencing nosocomial transmission such as patient age, antibody level, duration of exposure to the source of infection and viral genome type.

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Use of C-reactive protein to guide duration of empiric antibiotic therapy in suspected early neonatal sepsis

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Background. **Serial C-reactive protein (CRP) measurements have been shown to be useful for guiding duration of antibiotic therapy in neonates. This study sought to determine whether this is a safe and practical approach in a developing country.**

Methods. **The study was conducted at the Johannesburg Hospital between September 15, 1998, and January 15, 1999. Subjects included all neonates evaluated for suspected sepsis in the first 24 h of life who had negative initial and repeat CRP values (≤ 10 mg/dl). Repeat CRP measurements were performed between 24 and 48 h after birth. Antibiotic therapy was stopped in these infants at 24 to 48 h, and they were observed until 72 h, when the final blood culture results were available. The number of positive blood cultures in this group was determined.**

Results. **The repeat CRP estimation correctly identified 99 of 100 infants in the study as not requiring further antibiotic therapy (negative predictive value, 99%; 95% confidence intervals, 95.6 to 99.97%). The 1 infant with a positive blood culture was premature with a gestational age of 31 weeks. Eight babies required repeat evaluation for suspected sepsis, 4 presented on Day 3 to 4 and one of these babies died. All these neonates were of ≤33 weeks gestation.**

Conclusion. **The use of serial CRP measurements to guide antibiotic therapy is a safe and practical approach in neonates with suspected sepsis in a developing country.**

INTRODUCTION

Neonatal sepsis is a common problem with substantial morbidity and mortality. The early diagnosis of neonatal sepsis presents a clinical dilemma, because there is no single reliable method to distinguish which babies are actually infected from those with "suspected sepsis." A common method is to be guided by blood culture results after 48 to 72 h of incubation, by which time 98% of cultures ultimately yielding an organism will be positive.[1]

Automated blood cultures usually detect pediatric

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