Annals of Tropical Paediatrics (2001) 21, 59–65

Detection of adenovirus infection in children in Jordan

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(Accepted August 2000)

Summary Between November 1997 and May 1998, 350 nasopharyngeal aspirates (NPA) were obtained from children admitted to the Respiratory Disease Unit at Princess Rahma Hospital, northern Jordan who were clinically diagnosed as suffering from respiratory tract infections. NPA were investigated for the presence of adenovirus using shell vial (SV) culture assay, conventional culture (CC) assay, and direct immunofluorescence assay (DFA). Of the 350 NPA, adenoviruses were detected in 54 (15.4%) by the combined techniques used. SV identified 34 (63%), CC 48 (89%) and DFA 30 (56%). Most virus isolations were in children aged 1–<5 years old and were associated with pneumonia in 39% and bronchopneumonia in 32%. SV assay showed a sensitivity and specificity of 68.8% and 99.7%, respectively, for detecting adenovirus from NPA. These results emphasize that CC assay is still important for the diagnosis of adenovirus, although SV and DFA are superior diagnostic assays.

Introduction

World Health Organization (WHO) data indicate that respiratory tract infection (RTI) is one of the major causes of morbidity and mortality in infants and children.[1–3] In many countries, including Jordan, acute respiratory infection (ARI) accounts for nearly one-third of all deaths in children less than 5 years of age.[4,5] ARI kills an estimated four million children every year in developing countries and most of these deaths are caused by pneumonia.[6] In those countries, the rate of infection is very high, particularly in infancy and early childhood.[7]

Adenovirus is known to infect persons by aerosols or eye contact, but it also spreads by faecal/oral routes, especially where hygiene is poor. During an incubation period of 5–10 days, the virus replicates in pharynx, conjunctivae and small intestine. It is recognized that all adenovirus serotypes are endemic in the community and that for unknown reasons some can cause explosive outbreaks of disease, usually respiratory but also involving the eye.[8]

Adenoviruses are responsible for a significant number of clinical respiratory illnesses. Upper respiratory disease caused by adenoviruses includes colds, pharyngitis and tonsillitis and occurs mainly in infants and young children. Information about adenoviruses causing respiratory disease has been gathered in a series of reports to the WHO and evaluated over a 10-year period (1967–76),[9] during which time adenoviruses accounted for 13% of a total 135,702 reported cases. This was second only to influenza A, which accounts for 28% of reported cases.

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ISSN 0272-4936 print/ISSN 1465-3281/online/01/010059-07
Carfax Publishing
DOI: 10.1080/02724930020028939

Stratification by age of reported adenovirus cases revealed the following: 22% <1 year, 42% 1–4 years, 18% 5–14 years, 7% 25–29 years, 1% >60 years. Other studies indicate that approximately 10% of childhood pneumonia is probably caused by adenoviruses.[10] Other adenovirus-related lower respiratory illnesses include bronchitis and bronchiolitis.[11]

Adenoviruses are stable and do not need refrigeration during transport. They can be cultured and isolated in a variety of cell lines. Suitable cell lines include Hep-2, Hela, A549 and PMK.[12–14] The infected cells become swollen, rounded and refractive and, clustering together, form grape-like structures which subsequently become detached from their support.[10] Confirmation of infection is usually achieved by fluorescent assay, enzyme immunoassay or haemagglutination-inhibition.[14]

The purpose of this study was to determine the incidence of RTI caused by adenovirus and to evaluate the different diagnostic assays, shell vial (SV) culture assay, conventional culture (CC) assay and direct immunofluorescence assay (DFA), in terms of sensitivity, specificity, predictive value and agreement.

Patients and methods

Patients

This study involved 350 children less than 13 years old admitted with respiratory tract illness to the Respiratory Diseases Unit in the Princess Rahma Hospital. Those with congenital and persistent respiratory disease were excluded. Princess Rahma Hospital is a Ministry of Health paediatric teaching hospital administered by the Faculty of Medicine of Jordan University of Science and Technology. It is the only paediatric hospital in northern Jordan and serves Irbid city and suburbs. All information concerning patients was collected on a special proforma.

Specimen collection

Using the method described by Meziere *et al.*,[15] nasopharyngeal aspirates (NPA) were collected using a sterile infant feeding tube connected to a vacuum pump. The tube was introduced through each nostril, yielding about 0.5–1 ml of aspirate. The tube was placed into separate, tightly capped sterile containers labelled with the child's name, identification number and the collection date and time. The container was transported to the laboratory in wet ice.

Specimen processing

Specimens were processed according to the method described by Isenberg,[16] as follows. The contents of the infant feeding tube were emptied into an Ependorff tube by flushing with a mixture of 1.5 ml of PBS (pH 7.4) including 200 μl penicillin, 0.2 mg streptomycin and 10 μg amphotoricin B per ml. The specimen was vortexed for 30 s to dislodge cells from mucus and centrifuged at $400 \times g$ for 10 min using a Hettich centrifuge. The supernatant was aspirated and transported to another Ependorff tube for inoculation of cell culture. The pellet was used for rapid detection of adenovirus antigens by fluorescence assay.

Preparation of cell pellet for immunofluorescence staining

The cell suspension was prepared from the pellet by washing the pellet twice in PBS (pH 7.4) for 10 min to decrease the mucus viscosity. The sediment was then resuspended in 0.5 ml PBS. Twenty μl of cell suspension was spotted per well on an eight multi-well slide (5-mm diameter). The slide was dried by hotplate at 40°C and fixed with cold acetone at 4°C for 10 min. The slide was then stored in an airtight box at -70°C until used for staining.

Direct immunofluorescence staining

Ten μl of mouse monoclonal isothiocyanate-labelled anti-RSV antibody (Chemicon, USA)

was added to each well. The slide was incubated at 37°C for 30 min in a humid chamber and then rinsed for 10–15 seconds in PBS (pH 7.4). After mounting in phosphate-buffered glycerol (20–80% v/v), the slide was read using a fluorescence microscope (Nikon, Japan) at ×20–40 magnfication. Specimens yielding less than an average of 10 columnar epithelial cells per well were rejected. RSV-infected and uninfected human epithelial cells (Hep-2) were stained as positive and negative controls, respectively, in each trial. The presence of one positive columnar epithelial cell was required to consider a specimen positive for adenovirus antigen.

Virus isolation

Conventional culture (CC) assay. Specimen inoculation was done according to the method described by Isenberg,[16] as follows. A conventional culture tube with a semiconfluent monolayer of Hep-2 cells was inoculated by aspirating the old growth medium (GM) and delivering 0.2 ml of specimen supernatant to each tube. After adsorption at 37°C for 60 min, 1 ml fresh maintenance medium (MM) was added and the incubation continued for up to 14 days. The culture was maintained by replacing the old MM with fresh MM every other day. Culture tubes were examined daily for evidence of viral replication and development of CPE. When seen, CPE were harvested and prepared for immunofluorescence staining.

Shell vial (SV) culture assay. Shell vial assay was performed according to the method described by Olsen *et al.*,[13] as follows. A shell vial tube with semiconfluent monolayers of Hep-2 cells was inoculated by aspirating the old GM and delivering 0.2 ml of specimen supernatant to each tube. The specimen was centrifuged (700 × g) for 50 min (Hettich, Germany). After centrifugation, 1 ml of MM was added to each vial and incubated for 4 days. SV tubes were examined daily. When the SV tube showed toxic effects or contamination, the specimen was decontaminated and the culture repeated. At the end of the incubation period (regardless of the presence of CPE), the Hep-2 monolayers were harvested from the bottom of the SV tubes by scraping into a small volume of PBS, pH 7.4 by rubber policeman, and 20 μl of scraped cells were spotted onto each well of eight-well slides and prepared for immunofluorescence staining.

Data analysis

Groups were compared by means of the χ^2 test using Minitab version 11.

Results

A total of 350 specimens were tested for the presence of adenovirus by SV, CC or DFA. Table I shows the number positive for adenovirus by the assays used. The number positive for adenovirus by SV, CC and DFA was 54 (15.4%). Adenovirus was more frequently isolated with CC (88.9%) than with SV (63.0%) or DFA (55.6%).

The age and sex distributions of the positive and negative cases of adenovirus are shown in Table II. The children's ages ranged from 1 month to 13 years (mean 7 months). Data showed that adenovirus was highly associated with children aged 1–<5 years ($p < 0.001$).

Table III shows the findings in children positive for adenovirus. Adenovirus infection was associated with pneumonia in 38.9% and with bronchopneumonia in 31.5%. In this study, 75.9% of hospitalized children with adenovirus had received at least one type of antibiotic.

The frequency of signs and symptoms in the children positive for adenovirus is shown in Fig. 1. Cough and wheezing were the most prominent, 81.5% and 77.8%, respectively.

Comparison of SV with CC and DFA for isolation of adenovirus is shown in Tables IV and V. The sensitivity and specificity of SV compared with CC were 68.8% and 99.7%, respectively.

TABLE I. Number of adenoviruses positive by SV, CC or DFA assays in nasopharyngeal aspirates of 350 children

No. of positive adenovirus (%)	No. of specimens positive by SV	CC	DFA
54 (15.4)	34 (63.0)	48 (88.9)	30 (55.6)

TABLE II. Age and sex distribution of children positive for adenovirus by SV, CC or DFA assay

Age (yrs)	No. of children (M:F)	%	No. of positive children (M:F)	%
<1	236 (126:110)	67.4	19 (12:7)	8.1
$1-<5$	102 (60:42)	29.1	33 (18:15)*	32.4
5–13	17 (11:6)	4.9	2 (1:1)	11.8
Total	350		54	

* $p<0.001$.

TABLE III. Observations in 54 children positive for adenovirus by SV, CC or DFA assay

Observations	No. (%) of positive children
Clinical diagnosis	
Bronchiolitis	7 (13.0)
Pneumonia	21 (38.9)
Bronchopneumonia	17 (31.5)
Other respiratory infection (bronchitis, croup, upper respiratory tract)	9 (16.7)
Duration of hospitalization (days)	
1–3	23 (42.6)
4–6	22 (40.7)
7–9	3 (5.6)
No. of antibiotics received	
0	13 (24.0)
1	26 (48.1)
2	9 (16.7)
3	6 (11.1)

Discussion

This is the first study in Jordan to examine the role of adenovirus as an aetiological agent of RTI in children under 13 years of age. The nasopharyngeal aspirates were collected during the winter (November 1997 to May 1998) when RTI is more prevalent in northern Jordan.[17]

The infection rate of adenovirus varies from region to region and from one year to another,[8,10,18] and in this study it was higher than expected (15.4%). The rate of infection with adenovirus in children hospitalized during the winter months in Saudi Arabia was very low (0.7%).[19] The reported infection rate has ranged from 2 to 4% in hospitalized children in various developing countries[18] and a 6-year study revealed an only slightly higher infection rate of 8.2% among 1,416 sick children.[20] Our findings differ but agree with the reported infection rate of 14.3% among 169 hospitalized children in Argentina.[21] A similar study of 80 hospitalized children reported that the most important viral agent of RTI was RSV followed by adenovirus.[22] In addition to these two studies, data from WHO show that 13% of a total of 135,702 cases with respiratory illnesses were associated with adenovirus[9] and another study reports an adenovirus infection rate of 18% among 96 children hospitalized with respiratory illnesses.[23]

Our results indicate that adenovirus infection occurs mainly in children aged $1-<5$ years, in agreement with other reports.[21,22]

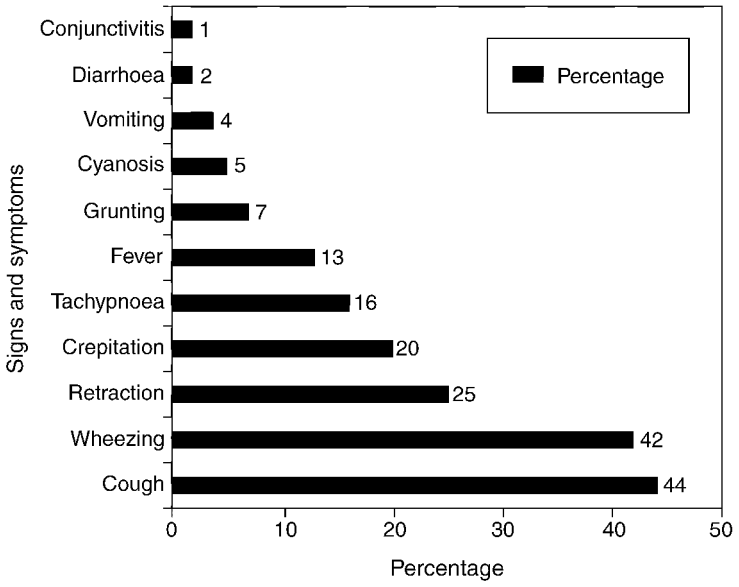

FIG. 1. The frequency of signs and symptoms in 54 children with adenovirus infection.

TABLE IV. Comparison of SV, CC and DFA for diagnosing adenovirus in nasopharyngeal aspirates of 350 children

Possible result	SV/CC	SV/DFA	CC/DFA
+/+	33	24	27
+/−	1	7	3
−/+	15	11	21
−/−	301	308	299

Adenovirus is responsible for a significant amount of clinical respiratory illness. Approximately 10% of childhood pneumonia is probably caused by adenovirus. Other lower respiratory illnesses related to adenoviruses include bronchiolitis and bronchitis.[9,10,24–26] A significant association was found between adenovirus and pneumonia in children older than 1 year.[21] Our study supports these findings in that adenovirus was identified in 38.9% children with pneumonia and in 31.5% with bronchopneumonia.

This study shows that 41 (76%) of our children received at least one type of antibiotic after admission. This exceeds the proportion reported in the literature. In one study, 61% of children were given antibiotics despite the fact that 35% of them had a confirmed viral aetiology.[22] The frequent use of antibiotics in our patients might reflect the fact that the Princess Rahma Hospital has no facility to test for viral infection and doctors might give antibiotics to avoid secondary bacterial infection.

Wide variation in the sensitivity of SV for

TABLE V. Percentage of sensitivity, specificity, predictive value and agreement of SV, CC and DFA in nasopharyngeal aspirates of 350 children

			Predictive value		
Assays compared	Sensitivity	Specificity	+	−	Agreement
SV/CC	68.8	99.7	97.1	95.3	95.4
SV/DFA	68.6	97.8	77.4	96.6	94.9
CC/DFA	56.3	99.9	90.0	93.4	93.1

detecting adenovirus has been reported. One study found that SV was approximately 80% sensitive for detecting adenovirus[26] while in another study SV assay using Hep-2 cell lines was only 52% sensitive at 24 hours for specimens previously known to be positive.[22] Furthermore, only 10 of 21 adenovirus isolates were detected by SV assay after 4 days incubation.[27] In one study comparing SV with traditional tube cultures using A-549 cell lines for detecting adenovirus, 77% of specimens were positive in SV at 2 days and 100% at 5 days post-inoculation, and most specimens were eye swabs.[28] It has been reported that SV works well when specimens contain relatively large amounts of the virus but are insensitive when specimens contain small amounts of the virus.[13] This might explain the high rate of detection of adenovirus in the earlier study.[28] Furthermore, the average time to detect adenovirus in specimens positive by SV was 4 days compared with 8 days for specimens negative in shell vial and positive only in CC.[13] In the present study, 33 (61 %) of 54 adenovirus isolates were detected by both SV and CC. However, 15 (28%) samples were positive by CC and negative by SV. Although SV assay is highly sensitive for detecting viruses, its sensitivity is only fairly good (69%) for detecting adenovirus. Based on our findings, it might be necessary to extend the incubation period of SV for isolating adenovirus or simply to use the CC assay. The variation in SV sensitivity for adenovirus could be owing to the use of different types of specimen; for example, throat or eye swabs yield higher numbers of adenovirus than nasopharyngeal specimens.[1,23] Low titres of viruses in specimens, especially adenovirus, make detection difficult.[1]

Our findings explain the importance of selecting the right laboratory assay to diagnose viral infection when managing children hospitalized with RTI. Positive laboratory diagnosis of respiratory viruses might persuade physicians to stop unnecessary antibiotic therapy because of its cost and side-effects and to shorten the duration of hospitalization; it might also reduce the need for other diagnostic tests.

Acknowledgment

Financial support for this research was provided by Jordan University of Science and Technology, grant no. 63/97.

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