

## Original Article

# Impact of Human Adenovirus Serotype 7 in Hospitalized Children with Severe Fatal Pneumonia in the Philippines

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(Received July 7, 2013. Accepted October 25, 2013)

**SUMMARY:** Human adenovirus (HAdV) serotype 7 is an important etiological agent of severe childhood pneumonia. The aim of this study was to define the role of HAdV7 and to describe its clinical and molecular epidemiological characteristics in the Philippines in 2011. HAdVs were detected by viral culture, and a partial region of hexon gene was sequenced. A total of 700 patients were enrolled, of which 22 (3.1%) died. Nine (1.3%) HAdV cases were confirmed, of which 7 were positive for HAdV7, 1 for HAdV3, and 1 for HAdV5. Among the 9 HAdV-positive cases, 4 (44%) with HAdV7 died. Molecular analysis revealed that all HAdV7 isolates were closely related to genome type h strains. This study demonstrated the significance of HAdV7 as an etiological agent of severe pediatric pneumonia with a high fatality rate. Hence, continuous monitoring is required to define the clinical and public health significance of HAdV7 infection.

## INTRODUCTION

Human adenovirus (HAdV) causes various serotype-specific diseases, such as acute respiratory infection, conjunctivitis, and acute gastroenteritis. The HAdV subspecies B1 includes serotypes 3, 7, and 21, which can cause lower respiratory infections (1,2).

Pneumonia is one of the most important causes of death in children under the age of 5 years, particularly in developing countries (3). Viruses, such as influenza virus, respiratory syncytial virus (RSV), and rhinovirus, are commonly associated with respiratory infections. HAdV also plays an important role in pediatric infections and accounts for 2%–5% of respiratory illnesses and 4%–10% of pneumonia cases (4). HAdV serotype 7 (HAdV7) is most significantly associated with severe pneumonia and causes fatal infections even in healthy children in developed countries (5). Furthermore, a variety of genome types can be identified by the restriction fragment length polymorphism (RFLP) technique (6). Temporal and geographical distributions of serotypes and genome types have been reported (2,5,7–12). It has also been indicated that the emergence or switching of serotypes and genome types can cause HAdV outbreaks (8).

In the Philippines, etiological studies on acute lower respiratory infection have been conducted, which identified HAdV as one of the several causative agents (13,14). However, to the best of our knowledge, de-

tailed analyses of HAdV surveillance coupled with molecular analysis has been limited in tropical countries (15). The present study was conducted to define the role of HAdV among severe childhood pneumonia cases and to describe the clinical and molecular characteristics of this virus in the Philippines.

## MATERIALS AND METHODS

**Patients:** This study was conducted at the Eastern Visayas Regional Medical Center (EVRMC) as part of a pediatric pneumonia study. The EVRMC is a tertiary governmental hospital located in Tacloban city, Leyte Island, Region VIII, the Philippines.

Nasopharyngeal swabs and blood samples were collected on the day of admission from patients aged 7 days to 14 years who were hospitalized with severe pneumonia. Written informed consent was obtained from parents or guardians. According to the Integrated Management of Childhood Illness guidelines (16), a case with severe pneumonia was defined as a child with cough or a difficulty in breathing and with any of the following signs: inability to eat, lethargy or unconsciousness, vomiting, convulsions, chest indrawing, or stridor.

Clinical and laboratory data were obtained by our research staff using a standard form. Rapid tests for identification of influenza virus and RSV were performed. Serum KL-6 glycoprotein levels were measured by a commercial diagnostic company (SRL, Tokyo, Japan), using an electrochemiluminescence immunoassay to evaluate the severity of interstitial pneumonia caused by HAdV.

**Viral isolation:** Nasopharyngeal swabs were transferred to the Research Institute of Tropical Medicine (RITM), Metro Manila, the Philippines. Fig. 1 shows a flow chart for detection of respiratory viruses. All sam-

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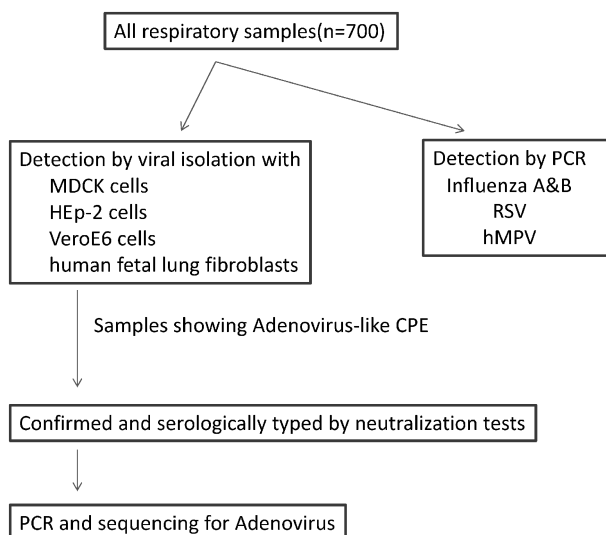


Fig. 1. Flow chart for detection of respiratory viruses.

ples were inoculated into Madin–Darby canine kidney (MDCK) cells, HEp-2 cells, VeroE6 cells, and human fetal lung fibroblasts. These cells were used to detect common respiratory viruses, such as influenza A and B viruses, enterovirus, human metapneumovirus (hMPV), RSV, and HAdV. When a cytopathic effect (CPE) was observed, the infected cells were harvested and kept frozen at  $-70^{\circ}\text{C}$  for future analysis. The samples showing HAdV-like CPEs were confirmed and serologically typed by neutralization tests with commercially available antibodies (Denkaseiken, Tokyo, Japan).

**Molecular methods:** DNA was extracted from all HAdV-positive isolates using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed using ExTaq DNA polymerase (TaKaRa Bio, Otsu, Japan), with specific primer sets (S29 + S52 and S51 + S53) (6), to amplify the region of the hexon gene corresponding to nucleotide position 289–1574 of HAdV3. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen). Sequence reactions were performed with fluorescent dideoxy chain-termination chemistry using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA). Sequences were determined using the Applied Biosystems 3500 Genetic Analyzer. In addition, PCR was performed to detect influenza A and B, RSV, and hMPV.

**Ethical approval:** The research protocol was approved by the ethics committee of Tohoku University Graduate School of Medicine (Sendai, Japan) and the institutional review board of RITM.

**GenBank accession numbers:** The determined nucleotide sequences were deposited in the GenBank/EMBL/DBJ databases under the accession numbers: AB742363–AB742371.

## RESULTS

In 2011, 700 patients with severe pneumonia were enrolled in this study, of whom 22 (3.1%) died. HAdV was isolated from 9 cases (1.3%) in at least one of the

cell lines for viral culture, of which 7 were identified as HAdV7, 1 as HAdV3, and 1 as HAdV5. Among the 9 HAdV-positive patients, 4 died due to severe pneumonia. HAdV7 was detected in all fatal cases.

Demographic and clinical information are summarized in Table 1. The patients' ages ranged from 27 days to 4 years. The range of HAdV7-positive cases was narrowed from 6 months to 2 years. Except for Case 2, no baseline disease or condition was recorded. Case 2 was recognized as malnutrition from emaciation. The respiratory symptoms in all patients began 2–10 days before hospitalization. Five patients took antibiotics before hospitalization. All fatal cases died within 3 days of hospitalization. Three of the 4 fatal cases received oxygen therapy through intubation. All cases received antibiotics intravenously. Case 6 was clinically diagnosed with a complication of central nervous system (CNS) infection.

Laboratory findings, white blood cell (WBC) counts, serum KL-6 levels, bacteria culture results, chest X-ray findings, and PCR results, are summarized in Table 2. WBC counts were 5,000–34,400 cells/ $\mu\text{l}$ , whereas the ratio of neutrophils and lymphocytes varied widely. Serum KL-6 levels showed clear differences between fatal and non-fatal cases, which ranged from 530–2,930 U/ml and 154–532 U/ml, respectively. However, the differences were not statistically significant ( $P = 0.11$ ). Bacteria, including *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Pseudomonas aeruginosa*, and *Moraxella catarrhalis*, were cultured from nasopharyngeal swabs. However, no bacteria were detected by blood culture. Chest X-ray findings of Cases 2, 3, and 7–9 showed bilateral interstitial shadows or patchy consolidations that were confirmed by radiologists. No lobar consolidation was revealed. The chest X-ray results were consistent with the findings of viral pneumonia. In Case 4, a small consolidation was shown in the right lung field, suggesting bacterial infiltration. Rapid tests for influenza and RSV were all negative. In addition, the results of PCR performed to detect the presence of influenza viruses A and B, RSV, and hMPV were all negative.

The hyper-variable regions of the hexon gene are the primary regions that determine the antigenicity of the neutralizing antibodies against each serotype (17). Therefore, the sequence of this region was analyzed. Except for Case 2, all HAdV7 isolates showed 100% homology to HAdV strains 87–922 (accession no. JN860676), which were detected in Argentina in 1987 and subsequently identified by RFLP as HAdV7 genome type h (HAdV7h). Case 2 had 1 synonymous mutation at position 298, which corresponded to the HAdV3 hexon gene. Isolation of HAdV3 and HAdV5 was confirmed using the same approach.

## DISCUSSION

HAdV is a significant causative pathogen of acute lower respiratory infection, which can cause severe pneumonia, resulting in death even in healthy individuals. Therefore, it is of great importance to decipher the clinical and molecular characteristics of HAdV. Although many studies have been published (2,5,18), data on the molecular epidemiology of HAdV in tropi-

Table 1. Demographic and clinical information of the HAdV-positive cases

Case no.	Patient ID/strain	Adenovirus serotype	Age	Sex	Baseline disease/ Past medical history	Symptoms duration prior to hospitalization	Pre-usage of antibiotics before hospitalization	Final diagnosis	Hospitalization period	Treatment O <sub>2</sub> /intubation	Treatment antibiotics	Other remark	Outcome
1	PHL/TTa164/2011	HAdV-7	7 m	F	none	7 days	+	pneumonia very severe	< 24 h	+ / + (day1)	+	convulsion	died
2	PHL/TTa227/2011	HAdV-7 <sup>1)</sup>	7 m	M	5 kg malnutrition	7 days	+	pneumonia very severe	< 24 h	+ / +	+	hypoxic seizure	died
3	PHL/TTa290/2011	HAdV-7	1 y 5 m	M	none	7 days	+	pneumonia very severe	3 days	+ / + (day2)	+		died
4	PHL/TTa407/2011	HAdV-7	9 m	M	none	10 days	none	pneumonia severe	6 days	none	+	bacterial pneumonia suspected	discharged
5	PHL/TTa410/2011	HAdV-7	2 y 0 m	M	none	7 days	+	pneumonia severe	5 days	none	+		discharged
6	PHL/TTa679/2011	HAdV-7	9 m	M	none	7 days	none	pneumonia very severe, CNS infection	2 days	none	+	CNS infection complicated	died
7	PHL/TTa696/2011	HAdV-7	6 m	M	none	4 days	none	pneumonia severe	9 days	none	+		HAMA improved <sup>2)</sup>
8	PHL/TTa230/2011	HAdV-3	4 y 4 m	F	pneumonia severe (0 y)	7 days	+	pneumonia severe	3 days	none	+		discharged
9	PHL/TTa208/2011	HAdV-5	27 d	M	none	2 days	none	pneumonia severe	11 days	none	+		discharged

<sup>1)</sup>: Synonymous mutation was observed.<sup>2)</sup>: HAMA, home against medical advice. Patient was discharged against medical advice, but later we confirmed he was recovered. Fatal cases are highlighted by shadow.

Table 2. Laboratory findings including white blood cell (WBC), serum KL-6, bacteria culture, chest X-ray remarks, and PCR results

Case no.	WBC		Serum KL-6*		Blood	Bacteria culture			Chest X-ray finding	PCR result Influenza A&B, RSV, hMPV
	/ $\mu$ l	Neutrophil (%)	Lymphocyte (%)	(U/ml)		Nasopharyngeal cavity 1	Nasopharyngeal cavity 2	Nasopharyngeal cavity 3		
1	17,800	81	16	1,240	No growth	<i>P. aeruginosa</i>			ND <sup>1)</sup>	negative
2	15,700	73	23	530	No growth	<i>H. influenzae</i> heavy growth	<i>Klebsiella</i> spp.		bilateral interstitial shadow, PM/SE <sup>2)</sup>	negative
3	5,300	59	36	1,140	No growth	No growth			bilateral patchy consolidation	negative
4	34,400	79	18	190	No growth	<i>S. pneumoniae</i> heavy growth	<i>M. catarrhalis</i> heavy growth		consolidation in right lung field	negative
5	5,000	56	38	297	No growth	<i>S. pneumoniae</i> heavy growth	<i>H. influenzae</i> heavy growth	<i>M. catarrhalis</i> heavy growth	ND <sup>1)</sup>	negative
6	6,400	72	22	2,930	No growth	<i>S. pneumoniae</i> moderate growth	<i>K. pneumoniae</i>		ND <sup>1)</sup>	negative
7	7,800	49	47	532	No growth	<i>Staphylococcus</i> spp. normal flora			bilateral interstitial shadow	negative
8	8,200	62	33	372	No growth	<i>S. pneumoniae</i> moderate growth	<i>H. influenzae</i> light growth		bilateral interstitial shadow	negative
9	14,500	31	58	154	No growth	<i>H. influenzae</i> heavy growth			bilateral interstitial shadow	negative

\*Serum KL-6 standard: children &lt;250 U/ml (23).

<sup>1)</sup>: ND, not done.<sup>2)</sup>: PM, pneumomediastinum; SE, subcutaneous emphysema.

Fatal cases are highlighted by shadow.

cal regions remain limited.

The EVRMC is a tertiary governmental hospital in the Eastern Visayas region of the Philippines. Treatment for pneumonia in the hospital is limited to basic supportive care, such as rehydration, antibiotic administration, oxygen therapy, and intubation. No mechanical ventilators are available at the EVRMC for pediatric patients and antiviral drugs are usually not administered even to cases with suspected viral infection such as influenza.

In the present study, HAdV was detected in 9 (1.3%) of 700 pediatric pneumonia cases, a rate that was lower than a previously reported rate of 4%–10% (4). The lower rate of HAdV detection may be due to several reasons. First, our study period was limited to 1 year and the study site was limited to a single institution. It is known that the incidence of HAdV varies between years and geographical locations. Second, our viral culture detection method may be less sensitive than PCR. Although the incidence was relatively low, HAdV infection was associated with a very high fatality rate of 44% (4/9). This rate was considerably higher than the fatality rate of 2.6% (18/691) of hospitalized patients with non-HAdV infections. Notably, all fatal cases were associated with HAdV7 infection, whereas patients with HAdV3 or HAdV5 infection were discharged. Our data suggested that HAdV7 infection caused more severe respiratory diseases than those due to other HAdV serotypes.

All HAdV patients were under 4 years of age, and particularly, all fatal cases involved patients who were less than 2 years of age. All of our patients were healthy children prior to infection, except for Case 2, who suffered from malnutrition. A fatal outcome among young healthy children was significant. All fatal cases began to show respiratory symptoms within 7 days prior to admission, and all died due to secondary respiratory failure within 3 days (2 cases within 24 h) of admission. These findings indicated the rapid deterioration caused by HAdV infection.

In consideration of our cases, together with the clinical symptoms and other laboratory results, we concluded that the isolated HAdVs derived from the nasopharyngeal cavity were the causative agents of pneumonia. However, Case 4 may have had bacterial pneumonia, considering the high WBC count, dominant neutrophil fraction, chest X-ray abnormality, and rapid effectiveness of antibiotics. Although Case 6 was suspected to have a complication of CNS infection based on the clinical symptoms, cerebrospinal fluid was not available for analysis. Except for Case 4, HAdV was likely to be the primary cause of pneumonia. Serum KL-6 levels are used to evaluate the state of interstitial pneumonia (19). All of the fatal cases showed considerably high KL-6 levels, although some of the surviving cases also showed high KL-6 values, compared with the standard value for children (19). Therefore, KL-6 levels may be associated with the severity of interstitial pneumonia caused by HAdV. This indicator could assist physicians to accurately diagnose disease severity.

Many molecular epidemiological studies of HAdV have been conducted, particularly for HAdV7, because of the high mortality rate. In addition, global spread and switching or displacement of the predominant ge-

nome types has been reported (10,20,21). A nationwide outbreak in 1995 in Japan was caused by HAdV type d2, which could have been imported from another Asian country. In South America, a switch from HAdV7c to 7h occurred in 1986, and HAdV7h has remained the dominant genome type, which has reportedly caused severe respiratory infections in Chile and Argentina (5,11,12,22). Although a higher virulence of HAdV7h was indicated during these outbreaks, there is no virological evidence to indicate increased virulence of HAdV7h. Outside of South America, HAdV7h was first identified in Japan in 1996 and then in the US in 1998 (5,9,23).

The present study showed that HAdV7 strains concordant to HAdV7h strains were prevalent in the Eastern Visayas region of the Philippines in 2011. The case fatality rate of HAdV7h was extremely high (44%) compared with the overall rate for all pneumonia cases. Carballal et al. (11) reported that the fatality rate of hospitalized HAdV7 patients reached 28.6%. Murtagh et al. (24) summarized 29 cases of acute lower respiratory diseases caused by HAdV7h between 1984 and 1988 in Argentina with a fatality rate of 34.5% (10/29). However, it is difficult to compare fatality rates among different studies conducted in different settings.

In conclusion, this study demonstrated that HAdV was a significant viral etiological pathogen, which can cause fatal pneumonia with a high fatality rate among children, particularly young infants. In the Philippines, there is no surveillance of HAdV infection; hence, the impact of HAdV infection may be underestimated. Therefore, sustained monitoring on the basis of molecular epidemiological methods is required to reveal background data of patients with HAdV infections to further develop public health strategies.

**Acknowledgments** This work was supported by a grant-in-aid for The Japan Initiative for Global Research Network on Infectious Diseases from the Ministries of Education, Culture, Sports, Science, and Technology, Japan, and a SATREPS Grant from Japan Science and Technology Agency and Japan International Cooperation Agency.

**Conflict of interest** None to declare.

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