Laboratory and Epidemiology Communications

Outbreak of Epidemic Keratoconjunctivitis Caused by Adenovirus Type 54 in a Nursery School in Kobe City, Japan in 2008

Kyoko Akiyoshi, Tomoko Suga, Kiyoka Fukui¹, Kiyosu Taniguchi², Nobuhiko Okabe³, and Tsuguto Fujimoto*²

Kobe Institute of Health, Kobe 650-0046; ¹Fukui Clinic, Kobe 653-0836; and ²National Institute of Infectious Diseases, Tokyo 162-8640, Japan

Communicated by Makoto Takeda

(Accepted June 13, 2011)

Human adenovirus type 54 (HAdV-54) is a novel type of adenovirus (1) that has so far been detected only in Japan, and causes epidemic keratoconjunctivitis (EKC) (1–3). HAdV-54 was identified as the HAdV-8 variant strain before 2008, because antibodies to HAdV-54 showed cross-reactivity with those to HAdV-8 in a neutralization test (NT) (1,3). In this report, we describe an outbreak of HAdV-54 conjunctivitis in a nursery school in Japan in 2008.

An outbreak of EKC occurred from 5 June to 13 August 2008 at a nursery school in Kobe City, Japan. On 5 June 2008, a 1-year-old nursery school child was referred to a local ophthalmologist for conjunctival infection, conjunctival swelling, and eye discharge. The patient was clinically diagnosed with acute allergic conjunctivitis. Since the disease is non-infectious, the child continued to attend nursery school (total number of students = 106). On 10 June, 5 students of the same class showed similar symptoms of conjunctivitis. Their parents (n = 4) also showed similar clinical manifestations on 17 June. Gradually, the conjunctivitis spread among the 3–5-year-old children in the other classes. For the purpose of investigation, an ophthalmologist (consultant) for the school interviewed several students and teachers on 30 June. After clinical examinations, the ophthalmologist diagnosed the disease as EKC. The patients were advised to stay in their homes to prevent further spread of the disease. The school authorities prevented the students and the teachers who were suspected to have EKC from attending school. However, many students who were incorrectly diagnosed with acute allergic conjunctivitis in the early stages of EKC at two ophthalmologic clinics continued to attend school. The number of patients increased daily, and at least 30 students of the nursery school (30/106, 28%) developed conjunctivitis. The incidence of EKC gradually decreased and finally terminated in mid-August, before the school closed for the Japanese summer holiday. In addition to the usual symptoms and signs of EKC, some students presented with fever. The acute symptoms of EKC waned within a month in all patients. However, 10–14 days after the onset of the disease, 8 patients (53.3%) developed corneal opacity. The corneal opacity persisted for a prolonged period in 2 patients, and these patients were still being treated as of 2010.

From 27 June to 30 July 2008, the ophthalmologist for the school collected conjunctival scrapings from 15 EKC patients: school students (n = 6), their family members (n = 6), school staff (n = 2), and others (n = 1). In 2000, we identified HAdV-8 in 2 isolates using NT (4), but these 2 isolates were observed to be HAdV-54 by Ishiko et al. who sequenced their complete genome (1). The complete genome sequence of HAdV-54 (Kobe-H strain) was deposited in GenBank (accession no. AB333801).

In 2008, 15 EKC samples were inoculated in FL, HEp-2, and Vero-E6 cells. Human adenovirus was isolated from 4 EKC patients. We sequenced the hexon-coding genes of the 4 strains. A PCR-sequencing method (5) was used with primers AdnU-S² and AdnU-A². The primer sequences were as follows: AdnU-S², 5'-TTCC CCC ATG GCN CAC AAY AC-3' where N = A/T/C/G and Y = T/C and AdnU-A², 5'-TGC CKR CTC ATR GGC TGR AGG TT-3', where K = G/T and R = A/G. An aliquot (2 µL) of the extracted DNA was used as the template. Amplification reactions were conducted using 50 µL of the reaction mixture that contained the following: each of the primer pairs, 0.5 µM; each dideoxynucleotide, 200 µM; SpeedSTAR™ HS DNA Polymerase (TaKaRa Shuzo, Shiga, Japan), 1 U; Tris-HCl (pH 8.0), 10 mM; and KCl, 50 mM. Forty cycles of PCR were performed, and each cycle consisted of the following incubations: 95°C for 5 s, 50°C for 15 s, and 72°C for 10 s. PCR products (554 bp) were purified using a QUiaquick PCR purification kit (Qiagen, Hilden, Germany) and used as templates for DNA sequencing reactions. Partial hexon gene sequences (350 bp) were determined and used to detect human adenoviruses types. The 4 isolates were identified as HAdV-54 because the sequences of the 4 strains were 100% identical to that of Adv-D54 (accession no. AB333801). Therefore, the PCR sequencing test is considered necessary for accurate identification of virus types.

The isolation rate of HAdV-54 was not high when compared to immunochromatographic test (ICT) kit,
Table 1. Thirteen laboratory confirmed cases of epidemic keratoconjunctivitis in this study

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Relationship to nursery school</th>
<th>Corneal opacity</th>
<th>Consequence of corneal opacity</th>
<th>Date of onset</th>
<th>Date of sample collection for virus isolation and PCR</th>
<th>Result of PCR isolation</th>
<th>Result of ICT 1</th>
<th>Result of ICT 2</th>
<th>Result of ICT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>m</td>
<td>school staff</td>
<td>opacity</td>
<td>opacity</td>
<td>2008/6/27</td>
<td>7/12 (1)</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>f</td>
<td>family of No. 4</td>
<td>opacity</td>
<td>opacity</td>
<td>7/2</td>
<td>7/12 (1)</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>1 y 1 m</td>
<td>m</td>
<td>student</td>
<td>opacity</td>
<td>opacity</td>
<td>7/11 (9)</td>
<td>7/11 (9)</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>1 y 2 m</td>
<td>m</td>
<td>family of student (aged 2)</td>
<td>opacity</td>
<td>opacity</td>
<td>7/11 (8)</td>
<td>7/11 (8)</td>
<td>negative</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>5</td>
<td>2 y 2 m</td>
<td>m</td>
<td>family of student</td>
<td>opacity</td>
<td>opacity</td>
<td>7/11 (3)</td>
<td>7/11 (3)</td>
<td>negative</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>f</td>
<td>school staff</td>
<td>opacity</td>
<td>opacity</td>
<td>7/11 (5)</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>m</td>
<td>family of No. 4</td>
<td>opacity</td>
<td>opacity</td>
<td>7/6</td>
<td>7/11 (5)</td>
<td>negative</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>m</td>
<td>family of No. 3</td>
<td>opacity</td>
<td>opacity</td>
<td>7/6</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>m</td>
<td>student</td>
<td>opacity</td>
<td>opacity</td>
<td>7/8</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>f</td>
<td>family of No. 4</td>
<td>opacity</td>
<td>opacity</td>
<td>7/8</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>m</td>
<td>student</td>
<td>opacity</td>
<td>opacity</td>
<td>7/8</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>12</td>
<td>1 y 2 m</td>
<td>m</td>
<td>family of No. 5</td>
<td>opacity</td>
<td>opacity</td>
<td>7/8</td>
<td>7/11 (5)</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>13</td>
<td>6 m</td>
<td>m</td>
<td>family of No. 5</td>
<td>opacity</td>
<td>opacity</td>
<td>7/20</td>
<td>7/23 (5)</td>
<td>negative</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
</tbody>
</table>

1): Among 15 patients, 2 patients were not shown in this table because adenovirus did not detected from them.
2): Pan-adenovirus PCR (6).
3): Number in parentheses indicates days after onset.

Because this virus seems to be fastidious like HAdV-8, HAdV-54 is a new type of human adenovirus that shows a cross-neutralization reaction with HAdV-8. In 2008, direct detection of the human adenovirus genome was conducted using the pan-adenovirus PCR method (6), which is not applicable for typing human adenovirus. This PCR method was used to examine clinical samples from 10 patients, and 8 samples (80%) showed positive results, including the same of 1 patient who showed negative results in the ICT. Two patients showed positive results in the ICT but showed negative results in the pan-adenovirus PCR test (Table 1). Samples for the PCR test were collected 6 or 15 days after those collected for the ICT. Late sampling from the 2 patients might explain their negative results in the PCR test. In 2009, we used PCR sequencing methods (5) and observed that the human adenoviruses isolated from clinical samples in 2008 were HAdV-54.

We had compared the sensitivity of the ICT kit and PCR in a previous study and found that PCR was more sensitive than the ICT kit (7). Thus, reexamination of the clinical samples using the PCR sequencing method was thought necessary to confirm the presence of HAdV-54 (5). However, only the isolates were stocked, and the clinical samples were discarded after diagnosis in 2008. Therefore, PCR sequencing for the typing of human adenovirus was possible only for the isolates in this study. This shows the importance of stocking clinical samples in such outbreak cases. We recommend direct detection of HAdV-54 genomes from clinical samples because isolation of HAdV-54 from clinical samples is difficult, as observed in this study. We detected the HAdV-54 genome in the samples of 66 EKC patients in Fukuoka Prefecture, Japan, using PCR and/or the newly developed loop-mediated isothermal amplification (LAMP) methods. However, we could not isolate any human adenovirus strains using the common virus isolation techniques (data not shown). Thus, we inferred that HAdV-54 is difficult to isolate. This might be explained by close nature of HAdV-54 to HAdV-8, which is known to be a fastidious virus.

Although acute follicular conjunctivitis followed by the development of subepithelial corneal infiltrates are important features of EKC, our report suggests that clinical presentation in the early stage of the HAdV-54 infection resembles that of acute allergic conjunctivitis and then subsequently shows the typical features of severe EKC. Two patients (13%) did not recover from severe corneal opacity. Both patients were adults and over 36 years old. They were treated using steroid eye drops. HAdV-54 has the potential to cause an outbreak of EKC in institutions and communities. In the early stage of the disease, it is frequently misdiagnosed as acute allergic conjunctivitis. Therefore, an early detection of HAdV-54 in a clinical setting is required. We recommend that an ICT for adenovirus should be performed in ophthalmology clinics to differentiate between the early stage of HAdV-54 EKC and acute allergic conjunctivitis. Laboratory confirmation using PCR is also recommended for the early detection of an HAdV-54 outbreak.

This article appeared in part in the Infectious Agents Surveillance Report (IASR), vol. 29, p. 346–347, 2008 in
Quarantine Station, Japan''

Rapid Test for Dengue Virus at the Narita Airport

Apropos “Evaluation of Nonstructural 1 Protein Rapid Test for Dengue Virus at the Narita Airport Quarantine Station, Japan”

Dear Editor: We comment on the laboratory data on 23 dengue-virus positive cases at the Narita Airport Quarantine Station (1) which indicated almost identical sensitivity of the dengue virus (DENV) NS1 antigen detection by the ELISA and the rapid test. The IgM/IgG detection employing either the antibody-capture ELISA or the rapid test was not found suitable to identify any DENV-carrying individuals. Our purpose is to share with clinicians and public health professionals the effectiveness of the combined rapid DENV NS1 and IgM/ IgG testing to detect cases with a primary or secondary virus infection during an outbreak.

The utility of a single-step immunochromatographic, one step dengue NS1 Ag and IgG/IgM test, (Dengue Duo; Standard Diagnostics, St. Ingbert, Germany) was immense during the 2010 DENV outbreak in Delhi, India. Among 175 suspected cases, 86 were NS1 positive and 89 were NS1 negative. Among 86 NS1-positive patients, 23 were IgM positive, 4 were IgG positive, and 6 were positive for all three markers. The 89 NS1-negative patients included 2 who were IgM positive, 8 who were IgG positive, and 7 who were positive both IgM and IgG: 72 were negative for all three markers and 53 patients were positive exclusively for NS1. Using Dengue Duo test, it was possible to diagnose 61 additional patients: these NS1 positives included 57 who were negative for IgM and 4 who were positive for IgG only (2).

Recently a novel serotyping NS1-ELISA has been described to identify the individual DENV serotypes (3). That format should be helpful during DENV screening. For example, among the 23 DENV infected cases at Narita Airport Quarantine Station, there were two DENV-2 and one DENV-4 infected cases that were positive by NS1 ELISA exclusively: one of these two DENV-2 cases was also IgM positive. Among them, a solitary DENV-2 and DENV-4 infection would have been missed by the combined rapid NS1 and the IgM/IgG test (1). In all probability, DENV-2 and DENV-4 infections should be picked up during the NS1-ELISA novel serotyping (3) ELISA or the rapid NS1 format.

In conclusion, a point-of-care format to detect DENV NS1 plus IgM and IgG concurrently is essential for diagnosis and DENV serotyping. Such a format would be useful for screening not only at the international airports but in resource-poor laboratories in several countries. The scenario in developing countries is alarming not only in rural and remote locations but also in urban areas (4).

Acknowledgments The secretarial assistance of Ms Sarita Kumar is acknowledged.

Conflict of interest None to declare.

Subhash C. Arya* and Nirmala Agarwal
Sant Parmanand Hospital, Delhi, India

REFERENCES

*Corresponding author: Mailing address: Sant Parmanand Hospital, 18 Alipore Road, Delhi-110054, India. E-mail: subhashbhapaji@gmail.com