Epidemic keratoconjunctivitis (EKC) mainly presents as acute follicular conjunctivitis with severe symptoms, such as serious conjunctival infection, severe discharge, formation of follicles and a pseudomembrane, and lacrimation. EKC is caused by the human adenovirus (HAdV). HAdVs are classified into seven species, from A to G (HAdV-A to HAdV-G). EKC is mainly caused by HAdV-8, HAdV-37, HAdV-53, HAdV-54, HAdV-56, and HAdV-64 (previously known as 19a), all of which are HAdV-D types. Recently, a new HAdV-D type, HAdV-85, was reported as a candidate variant associated with EKC (1). EKC is a contagious conjunctivitis associated with community-acquired infection, with more than 500,000 EKC cases reported annually by the National Surveillance Center in an endemic year. Therefore, in Japan, the study of EKC is often performed in eye clinics by ophthalmologists who are specialists in infectious eye diseases, and HAdV types are identified using conjunctival samples from patients with suspected EKC. HAdV-Ds were mostly determined from samples in previous reports (Table 1) (2–6). HAdV-B and -E were also identified at lower frequencies, as there are cases for which the differences between EKC and other adenoviruses, such as pharyngoconjunctival fever (PCF), are difficult to determine. During an EKC study in 2018, we observed two cases of conjunctivitis caused by HAdV-2 (an HAdV-C type) variant strains, which were treated at two eye clinics in different parts of Japan. The study was ethically reviewed and approved by the National Institute of Infectious Diseases (number 708).

Case 1: A 34-year-old man developed severe conjunctival injection, with a 2-day history of discharge and lacrimation, and he visited a neighborhood eye clinic in June 2018 in Fukushima city, in northern Japan. Examination revealed acute follicular conjunctivitis, subepithelial corneal infiltrates, and punctate keratitis. No other systemic symptoms, such as fever, pharyngitis, and preauricular lymphadenopathy, were observed. Testing with the immunochromatography (IC) kit (FUJI DRI-CHEM IMMUNO AG Cartridge Adeno OPH, FUJIFILM Corporation, Tokyo, Japan) for the rapid detection of adenoviral antigens using conjunctival samples revealed positive results. The clinical course that followed remained unknown, as he was only examined once.

Case 2: A 9-year-old boy developed moderate conjunctival injection, with a 5-day history of discharge and eyelid swelling, and he visited a neighborhood eye clinic in July 2018 in Tokyo, the capital of Japan. Examination revealed moderate acute follicular conjunctivitis. No corneal disorders were observed. As in Case 1, no other systemic symptoms were observed. The conjunctival samples yielded positive test results using the same IC kit as in Case 1. After five days, the symptoms of conjunctivitis had improved.

Table 1. Human adenovirus species and types identified in past EKC studies in Japan

| HAdV species and type | Year | References |
|-----------------------|------|------------|
| B3, B7, B11           | 1980–2000 | 2         |
| E4                    |      |            |
| D8, D64/19, D37       |      |            |
| B3, B11, E4           | 2005–2006 | 3         |
| D8, D37               |      |            |
| B3                    | 2003  | 4          |
| E4                    |      |            |
| D8, D64/19, D37, D53, D54 | | |
| B3                    | 2000–2009 | 5         |
| E4                    |      |            |
| D8, D64/19, D37, D53, D54 | | |
| D37, D54, D56         | 2008–2009 | 6         |

1): HAdV-19 was renamed as HAdV-64.
The extract solution remaining from the IC kit in both cases was used for the detection and quantification of HAdV DNA using real-time polymerase chain reaction (PCR), as described previously (7). HAdV DNA copy numbers are shown in Table 2. For HAdV typing, nucleotide sequences in the hexon, which contains the neutralizing epitope, were amplified (8) and subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/blast/). Both samples were identified as HAdV-2. During this study, HAdV-53, -54, and -85 were also identified from other conjunctival samples.

As a form of conjunctivitis caused by HAdV other than EKC, PCF is an acute and highly infectious illness characterized by fever, pharyngitis, and acute follicular conjunctivitis. Symptoms of PCF-related conjunctivitis are generally milder than those of EKC. Furthermore, moderate adenoviral conjunctivitis without systemic symptoms (i.e., non-EKC and non-PCF) was also observed. These cases of adenoviral conjunctivitis could have been caused by HAdV types other than HAdV-D.
Some of these PCF and simple to moderate adenoviral conjunctivitis cases are difficult to distinguish from EKC; this is true even for specialists in infectious eye diseases. Therefore, HAdV-3, -7, -11 (all HAdV-B types) and HAdV-4 (an HAdV-E type) were also identified at lower frequencies in the previous EKC study by ophthalmologists specializing in infectious eye diseases. However, HAdV-2 was not identified at all in Japan (Table 1).

HAdV-2 is classified as HAdV-C together with HAdV-1, -5, and -6. HAdV-C types primarily cause respiratory infections, such as fever, pharyngitis, rhinitis, and cervical adenopathies. HAdV-2 is one serotype known to cause PCF, and it has been isolated from conjunctival samples. However, severe conjunctivitis due to HAdV-2, such as EKC, has not yet been reported, and HAdV-2-associated conjunctivitis was not identified in the EKC study in Japan. The two cases in this study showed positive test results using the IC kit and PCR with a high amount of DNA compared with other cases of EKC caused by HAdV-D. Case 1 is thought to have been EKC because of the severe conjunctivitis, while Case 2 might have been moderate adenoviral conjunctivitis.

For the complete genome sequence, recombinant HAdVs between different HAdV types were recently reported in Japan as new genotypes from conjunctival samples, such as HAdV-53, -54, -56, and -85 (1,5,6). The emergence of new HAdV types often causes EKC outbreaks, which prevents many students from attending school due to the School Health Law in Japan.

Moreover, new HAdV types often cause nosocomial EKC infections during severe outbreaks in ophthalmology wards (5). Nosocomial EKC infections force restriction of clinical practice, and they have become a serious social problem in Japan.

The results of additional phylogenetic analyses revealed that the nucleotide sequences of the hexon and fiber genes in these two cases were identical to those in HAdV-2; therefore, these formed the same cluster as HAdV-2 (Figs. 1A and 1B). However, phylogenetic analysis of the penton bases showed that these two cases formed the same cluster as HAdV-1, not HAdV-2 (Fig. 1C). The GenBank/EMBL/DDBJ accession numbers of the nucleotide sequences presented in this study are LC496102–LC496107. Thus, these might be HAdV-2 variants suggesting recombination. Similar HAdV-C strains were identified from respiratory tracts and feces in China (9) and Germany (10), respectively. The relationship between these two HAdV-C strains and HAdV-2 variants was not evaluated in this study. Case 1 experienced severe conjunctivitis, which showed EKC-like symptoms, and this HAdV-2 variant is likely to be the cause of EKC. The diagnosis of EKC with moderate symptoms is obscure and depends on the discretion of the individual ophthalmologist. The epidemiology of conjunctivitis caused by the HAdV-2 variant should be carefully monitored using accurate EKC diagnostic criteria in the future.

Conflict of interest None to declare.

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