

## Is It Possible to Detect the Presence of Adenovirus Antigen in Conjunctiva Before the Onset of Conjunctivitis?

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**Abstract :** Background : The presence of adenovirus (Ad) in the conjunctiva was analyzed to investigate the potential of the eye in the incubation period as a source for spreading infection. Materials and Methods : Conjunctival scrapings obtained from the first infected eye and the second eye before and after the onset were evaluated by an immunochromatography test and the polymerase chain reaction method to detect Ad antigen. Results : Although bilateral cases were observed in 72%, no specimens obtained before the onset showed a positive result for Ad in both tests. Conclusion : These results suggest the possibility of the eye in incubation period as being a source for spreading infection is considered to be very small.

**Key words :** Adenovirus, Conjunctivitis, Incubation period, PCR

### Introduction

Adenoviruses have been implicated in a wide range of clinical diseases affecting mainly the respiratory, ocular and gastrointestinal systems. Ocular adenoviral infections have a variety of clinical presentations : including epidemic keratoconjunctivitis (EKC), pharyngoconjunctival fever (PCF) and nonspecific follicular conjunctivitis. Epidemics frequently occur and may be propagated by eye clinics. Transmission is by direct or indirect contact with ocular or upper respiratory tract secretions.

The incubation period ranges from 2 days to 2 weeks and infected people remain contagious for up to 2 weeks after the symptoms begin.<sup>1)</sup> Controversy remains whether patients with adenoviral conjunctivitis in the incubation period are

contagious. These patients can be observed among those who have suffered from bilateral adenoviral conjunctivitis especially in hospital infections. It would be clinically important to show whether if the presence of adenovirus (Ad) antigen is demonstrated by laboratory diagnostic tests in the conjunctiva before the onset of conjunctivitis. The Ad infection in the course of allogeneic stem cell transplantation is associated with a high transplant-related morbidity and mortality. Disseminated Ad disease is lethal in most instances. The early detection of Ad infection and identification in the peripheral blood of patients by real-time PCR method may thus serve as a basis for the early initiation of preemptive antiviral treatment, but it remains a major challenge due to the need to procure various laboratory instruments.<sup>2)</sup>

In this study, we analyzed the presence of Ad in the conjunctiva of eyes in incubation period (sec-

ond eye) in patients with bilateral adenoviral conjunctivitis before the onset of the second eye by an immunochromatography (IC) test and polymerase chain reaction (PCR) to investigate the potential of the eye in incubation period as a source for the spread of infection.

### Materials and Methods

The study population consisted of 32 patients with a mean age of 38.4 years who were diagnosed to have adenoviral conjunctivitis. The clinical data of all patients whose specimens were included in this study were examined retrospectively to investigate the correlation between the results of laboratory testing and the clinical diagnosis. The diagnosis was confirmed by the clinical findings and the positive result of PCR-restriction fragment length polymorphism (RFLP) analysis method for Ad antigen in conjunctival scrapings as described previously<sup>3)</sup> obtained on their first visit to the outpatient clinic to also determine the serotypes of Ad. The IC test (Adenocheck, SA Scientific Inc., San Antonio, TX), a sandwich immunoassay composed of a combination of both monoclonal and polyclonal antibody reactions to Ad performed on a paper membrane, was also carried out on the conjunctival scrapings. The details of this rapid diagnostic kit and its characteristic with regard to sensitivity, specificity and technical complexity have all been described by our group previously.<sup>4)</sup> The patients were directed to visit the outpatient clinic at approximately 3 days intervals until the onset of the second eye or 2 weeks after the onset of the first eye, and conjunctival scrapings were obtained and tested repeatedly by both IC and PCR methods to determine the presence of Ad in the conjunctiva of the second eye. The clinical severity of conjunctivitis was classified as mild, moderate and severe, according to the previously established criteria.<sup>5)</sup>

### Results

The serotypes of 32 patients with adenoviral conjunctivitis determined by PCR-RFLP method were as follows ; Ad type 37 (Ad37) (24 cases), Ad8(4), Ad3(3) and Ad7(1). Out of the 32 cases, twenty-three cases (72%) developed conjunctivitis in the second eye. Among these bilateral cases, 18 cases

(18/23 ; 78%) demonstrated a positive finding for Ad by the PCR method after the onset (Table 1). Thirteen of 18 cases were also IC-positive (sensitivity=72%) using specimens from the second eye (Table 1). No patients without any signs of acute conjunctivitis in the second eye (unilateral cases) showed a positive result for Ad on any occasions. However, no specimen from the second eye obtained before the onset showed a positive result either by PCR or IC. The latest occasion of sample collection in these cases ranged between 1 and 4 days as shown in Table 2. The serotypes of Ad detected from the second eye were identical to those from the first eye in all cases as follows ; Ad37(14), Ad8(1), Ad3(2), Ad7(1). The relationship between the clinical severity of conjunctivitis in PCR-positive-bilateral cases (18 cases) is displayed in Table 3. In general, the second eye involvement was clinically equal or milder in comparison with the first eye lesion in all cases.

### Discussion

Ad is a common ocular pathogen that frequently induces sporadic and sometimes an epidemic infection. Therefore, several rapid diagnostic tests<sup>4)6)</sup> and laboratory diagnostic methods<sup>3)</sup> have been developed. The practical implications of these observations are that physician, medical staff and family members can be informed prospectively about the expected spread and clinical pictures of the illness. In our study, the presence of Ad antigen was not demonstrated from the second eye of patients with adenoviral conjunctivitis before the onset. This finding suggests the possibility that the eye in the suspected incubation period of patients with adenoviral conjunctivitis as a source for the spread of infection is very small. However, this possibility cannot be excluded because of the following reasons. The minimal interval of sample collection before the onset in our cases series was 1 day. However, if conjunctival scrapings could be obtained within one day before the onset, then Ad antigen might be detected by laboratory tests. The detection limit of Ad DNA of PCR-RFLP method used in this study was reported to be  $10^6$  virus particles after the first amplification and  $10^3$  particles after two-step amplifica-

**Table 1.** Results of the Virological Test in Patients with Bilateral Conjunctival Involvement

| Case | Primary side |                   | Results of the latest PCR test and onset of the second eye |   |   |   |   |    |    |    |    |    |    |    |    |    |      | Secondary side |                 |                   |
|------|--------------|-------------------|--|---|---|---|---|----|----|----|----|----|----|----|----|----|------|----------------|-----------------|-------------------|
|      | Serotype     | Clinical severity | Day after the onset of the primary eye                     |   |   |   |   |    |    |    |    |    |    |    |    |    |      | Serotype       | Detection by IC | Clinical severity |
|      |              |                   | 5  | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |      |                |                 |                   |
| 1    | 37           | moderate          | —  |   | * |   |   |    |    |    |    |    |    |    |    |    |      | 37             | +               | mild              |
| 2    | 37           | severe            |  |   |   |   |   | —  |    | *  |    |    |    |    |    |    |      | 37             | +               | severe            |
| 3    | 3            | mild              | —  |   |   | * |   |    |    |    |    |    |    |    |    |    |      | 3              | +               | mild              |
| 4    | 37           | moderate          |  |   |   |   |   | —  | *  |    |    |    |    |    |    |    |      | 37             | —               | moderate          |
| 5    | 37           | moderate          |  |   |   |   |   |    |    | —  |    | *  |    |    |    |    |      | 37             | +               | mild              |
| 6    | 37           | severe            | —  |   |   | * |   |    |    |    |    |    |    |    |    |    |      | 37             | +               | moderate          |
| 7    | 37           | mild              | —  |   | * |   |   |    |    |    |    |    |    |    |    |    | n.d. | —              | mild            |                   |
| 8    | 37           | moderate          |  |   |   |   |   |    |    |    |    |    |    |    | —  | *  | 37   | +              | mild            |                   |
| 9    | 37           | severe            | —  |   |   | * |   |    |    |    |    |    |    |    |    |    | 37   | +              | mild            |                   |
| 10   | 37           | moderate          | —  |   |   |   |   | *  |    |    |    |    |    |    |    |    | n.d. | —              | moderate        |                   |
| 11   | 7            | moderate          |  |   |   |   |   |    | —  | *  |    |    |    |    |    |    | 7    | +              | mild            |                   |
| 12   | 8            | severe            |  |   |   |   |   |    |    |    | —  |    |    |    |    |    | 8    | —              | moderate        |                   |
| 13   | 37           | severe            |  |   |   |   |   | —  |    |    |    | *  |    |    |    |    | 37   | +              | severe          |                   |
| 14   | 37           | moderate          |  |   |   |   |   |    | —  |    | *  |    |    |    |    |    | n.d. | —              | mild            |                   |
| 15   | 37           | moderate          | —  |   |   | * |   |    |    |    |    |    |    |    |    |    | 37   | +              | moderate        |                   |
| 16   | 3            | mild              | —  |   | * |   |   |    |    |    |    |    |    |    |    |    | 3    | +              | mild            |                   |
| 17   | 37           | severe            | —  |   | * |   |   |    |    |    |    |    |    |    |    |    | 37   | —              | mild            |                   |
| 18   | 37           | moderate          |  |   | — |   |   |    | *  |    |    |    |    |    |    |    | 37   | +              | moderate        |                   |
| 19   | 37           | severe            |  |   |   |   |   |    |    |    |    |    |    |    | —  | *  | 37   | —              | mild            |                   |
| 20   | 37           | moderate          |  |   |   | — |   |    |    |    | *  |    |    |    |    |    | n.d. | —              | moderate        |                   |
| 21   | 8            | severe            |  |   |   |   |   | —  |    | *  |    |    |    |    |    |    | n.d. | —              | moderate        |                   |
| 22   | 37           | severe            | —  |   |   |   |   | *  |    |    |    |    |    |    |    |    | 37   | +              | moderate        |                   |
| 23   | 3            | mild              | —  |   |   |   |   | *  |    |    |    |    |    |    |    |    | 37   | —              | mild            |                   |

— : negative result, \* : onset of the second eye, n.d. : not detected (PCR negative), IC : immunochromatography, + : positive result.

**Table 2.** Relationship Between the Days of Specimen Collection in the Second Infected Eye Before the Onset and the Adenovirus Detection Rate in Two Methods Using the Specimens Obtained After the Onset

| Days of specimen collection* | Number of cases | Method of adenovirus detection and the number of positive cases using the specimens obtained after the onset |     |
|------------------------------|-----------------|--|-----|
|                              |                 | IC   | PCR |
|                              |                 | 1  | 3   |
| 2                            | 10              | 5  | 7   |
| 3                            | 7               | 5  | 6   |
| 4                            | 3               | 1  | 2   |
| Total                        | 23              | 13   | 18  |

\* Days are indicated as the day before the onset of the second eye.

**Table 3.** Relationship Between the Clinical Severity of Conjunctivitis in the First and Second Infected Eyes

|           |          | Second Eye |          |        |       |
|-----------|----------|------------|----------|--------|-------|
|           |          | Mild       | Moderate | Severe | Total |
| First Eye | Mild     | 3          | 0        | 0      | 3     |
|           | Moderate | 4          | 3        | 0      | 7     |
|           | Severe   | 3          | 3        | 2      | 8     |
|           | Total    | 10         | 6        | 2      | 18    |

tion.<sup>3)</sup> In contrast, the detection limit of the current real-time PCR method for the detection of Ad has been reported to be 116 copies per LightCycler capillary.<sup>7)</sup> Therefore, there is a possibility that if the virus amount was more than the detection limit of real-time PCR, then, Ad DNA could be detected before the onset of conjunctivitis in the second-infected eye even though the amount was less than that used for the PCR-RFLP method. The second reason is that since the clinical severity of the second eye was milder than the first eye, the volume of the virus particles in the conjunctiva might be less in the second eye than in the first eye. Neutralization antibody against Ad produced in the serum after the onset of the first eye might also have an inhibitory effect on detection of Ad antigen in the second eye. The variation of viral growth speed among Ad serotypes<sup>8)</sup> should also be considered as an important factor in the management of spread of infection of this disease, since there is a possibility that a fast growing strain of Ad may induce infectivity before the onset of conjunctivitis. This might be supported by the fact that Ad type 37 (cases 8 and case1<sup>9)</sup> and type 8 (case 12) were detected from the patients who had presumed incubation period of longer than 2 weeks possibly due to the slow growth speed of Ad subgenus D.<sup>6)</sup> However, it should be noted that positive rate of PCR for Ad in the second eye (78%) was higher than that in the first eye (72%), thus indicating that the symptomatic second eye may thus have a similar contagiousness to that in the first eye.

It has been widely accepted that patients with adenoviral conjunctivitis remain contagious approximately as long as 14 days after the onset, and based on this, patients are usually informed to take leave from work or school for a considerable period of time. If they need to be hospitalized due to any special reason, such as post-surgical care and observation, etc., they might also thus be isolated in a special restricted area to prevent contact with others. The incubation period of the second eye in general cases was 7 to 12 days as shown in our study. However, in some cases, the presumed incubation period may extend more than 14 days as observed in cases 8, 12 and 19 (Table 1). Controversy remains whether special clinical precaution

is necessary in these cases with a considerably long incubation period. In addition, in some cases, an adenoviral infection may prevent the induction of conjunctivitis in the second eye (unilateral case). The rate of unilateral cases was 28% in this study. Although these preventive measures are effective and clinically important, based on the results of this study, we also should pay attention not to negatively affect the patients' quality of life by taking excessive precautions such as those against the second infective eye. Further study on the virological kinetics of Ad in the human conjunctiva in clinical situations is called for.

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