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# Molecular Epidemiology of Adenovirus Strains Genotypes among Patients with Acute Follicular Conjunctivitis

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**Abstract** **Background:** Adenoviruses are a group of viruses that can infect the membranes (tissue linings) of the respiratory tract, eyes, intestines, and urinary tract. Conjunctivitis is the inflammation of the clear inside lining of the eyelids and the outer layer of the sclera and is commonly referred to as "pink eye." It can have either infectious or non-infectious causes. A common cause of infectious conjunctivitis is by the DNA virus Adenovirus which consists of several different serotypes between 65% and 90% of cases of viral conjunctivitis are caused by adenoviruses, and they produce two of the common clinical entities associated with viral conjunctivitis, pharyngoconjunctival fever and epidemic keratoconjunctivitis., whereas epidemic keratoconjunctivitis is more severe and presents with watery discharge, hyperemia, and ipsilateral lymphadenopathy. Adenovirus is highly contagious, so multiple cases are common in close-contact settings .The types of adenovirus causing pinkeye may be transmitted by water (in lakes and swimming pools), by sharing contaminated objects (such as towels or toys), or by touch. Patients should avoid touching their eyes, shaking hands, and sharing towels, among other activities. Transmission may occur through accidental inoculation of viral particles from the patient's hands or by contact with infected upper respiratory droplets, fomites, or contaminated swimming pools. **Purpose:** To find out the role and molecular epidemiological feature of adenovirus as etiologic agent in acute conjunctivitis in Saudi Arabia, In addition highlight the importance of implementing the proper infection control practices in ambulatory care. **Methods:** This was a prospective observational study conducted at outpatient clinic at one of Specialized Eye Hospital in Jeddah Kingdom of Saudi Arabia. Dates of the study were from October 2013 till April 2014. This study included 404 patients with acute follicular conjunctivitis presented to outpatient clinic. All specimens were examined by direct immunofluorescence antibody test for adenovirus. The positive specimens were then subjected for PCR genotyping. **Results:** Adenovirus was detected in 83 patients out of 404 (20.5%). 14 cases out of 83 (16.8%) were healthcare associated infection. The percentages of different serotypes of adenovirus were as follows; the predominant serotype was Ad4 24 out of 83 (28.9%), then Ad19 was 19 out of 83 (22.8 %), whereas Ad8, Ad37, Ad11, Ad3 and were identified in 8.4%, 4.8%, 3.6 %, and 3.6% respectively. **Conclusion:** This study confirmed the endemicity of adenoviruses as etiological agent of acute conjunctivitis, You can protect yourself and others from adenoviruses illnesses by Wash your hands often with soap and water &, keep surfaces clean, &strictly follow infection control practices.

***Keywords:*** *Molecular epidemiological, follicular conjunctivitis, immunofluorescence, endemicity, Adenovirus, PCR genotyping*

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## 1. Introduction

Adenoviral conjunctivitis accounts for 65-90% of viral conjunctivitis. This is a highly infectious condition (incubation: 3-29 days, infectious for a further 2 weeks) which can range from mild to severe. [1]

Ocular adenoviral infections exhibit varieties of clinical presentations, such as epidemic keratoconjunctivitis (EKC), pharyngoconjunctival fever (PCF), nonspecific follicular conjunctivitis, and chronic papillary conjunctivitis [2]. EKC is characterized by severe bilateral conjunctivitis with corneal involvement. The incubation period is 8-10 days. Corneal affection may persist for months. Although not blinding, the ocular infections create discomfort that leads to decreased quality of life and even possibly economic damages. [3] Adenoviral keratoconjunctivitis is transmitted through direct contact with the infected area and frequently causes community epidemics. The patients are infectious during the first 2-3 weeks of infection. Infection routes can include contaminated towels, and other contaminated articles of daily use in kindergartens, schools, and swimming pools. Clearly identified risk factors for infection include contaminated ophthalmological instruments or solutions, and insufficient hand hygiene.

To prevent transmission and outbreaks appropriate disinfection of ophthalmological instruments and hands should take place. [4,5] Epidemic keratoconjunctivitis: this refers to adenoviral infection involving the cornea. This condition is more severe with formation of subepithelial corneal infiltrates and pseudomembranes. Patients may have photophobia and reduced vision long after the conjunctivitis settles [6] diagnosis of ADV with more rapid diagnosis can be made by direct detection methods such as enzyme immunoassay, radioimmunoassay, and direct immunofluorescent antibody test. it offers a rapid and specific method for the direct detection of adenovirus in clinical smears. Yet, the sensitivity of this test is not very high, and a negative test does not always rule out the possibility of adenoviral infection. Recent development of PCR – based rapid serotype identification offer a sensitive and specific test for detection of common adenovirus strains [7,8].

## 2. Material and Methods

History and Focused ocular examination were done. Conjunctival swabs were taken from patients with acute follicular conjunctivitis within seven days of onset of symptoms who presented to Eye Hospital during the period from October 2013 to April 2014.

### 2.1. Exclusion Criteria

Newborn, or age less than 3 moth or patient presented with discharge of pus to exclude gonococcus conjunctivitis and chlamydia conjunctivitis, or patient with allergic conjunctivitis usually diagnosed by an eye doctor during an eye exam.

### 2.2. Specimen

Local anesthetic eye drop (Benoxinate hydrochloride 0.4%) was applied to the conjunctival sac. The upper and lower conjunctival surfaces were wiped with two cotton swabs vigorously. The swab was rotated during sampling process to ensure that the entire conjunctival surface was sampled. One swab was used for IMAGEN™ adenovirus test. The other one was collected in 1 ml of phosphate buffered saline (PBS) for PCR. These samples were preserved at - 30°c until the analyses.

IMAGEN™ Adenovirus is a qualitative direct immunofluorescence test for the detection of adenovirus in clinical samples [9].

### 2.3. PCR

The positive samples were then preceded for PCR Genome typing, tested positive for HAdV, were subjected to the molecular genotyping of HAdV by DNA sequencing.

DNA extraction was performed using the QiaAmp DNA mini kit (Qiagen 51304), according to the manufacturer's instructions. (ABI 3130; Applied Biosystems, Inc., USA [10].

## 3. Results

This study included 404 patients with acute follicular conjunctivitis presented to outpatient clinic at Eye hospital from October 2013 until April 2014 (Table 1). Their age range was from 3 months-46 years (Table 3). 300 patients were male and 104 were female. The percentage of adenovirus was 83 out of 404 (20.5%) detected by direct immunofluorescence test. 14 out of 83 (15.8%) were health care associated infection (Table 1). The different types of adenoviruses detected analysis were shown in Table 4.

**Table 1. Cases distribution throughout the study period**

| MONTH | Number of patients with conjc. | Positive infection with adenovirus | PERCENTAGE |
|---|---|---|---|
| October | 59 | 5 | 8.4% |
| November | 73 | 6 | 8.2% |
| December | 44 | 7 | 15.9% |
| January | 66 | 17 | 25.75% |
| February | 51 | 16 | 31.3% |
| March | 49 | 22 | 44.8% |
| April | 62 | 10 | 16.1% |
| TOTAL | 404 | 83 | 20.5% |

**Table 2. Cases distribution of health care associated infection and community acquired infection**

| Positive infection with adenovirus | HAI infection | Community acquired |
|---|---|---|
| 5 | 0 | 5 |
| 6 | 0 | 6 |
| 7 | 2 | 5 |
| 17 | 6 | 11 |
| 16 | 4 | 12 |
| 22 | 2 | 20 |
| 10 | 0 | 10 |
| 83 | 14 | 69 |

**Table 3. Age distribution among patients with adenoviral conjunctivitis**

| Age | No. of patients |
|---|---|
| 3-11 months | 6 |
| 1-4years | 16 |
| 5-14years | 32 |
| >14 years | 29 |
| Total | 83 |

**Table 4. Percentages of adenovirus typing analyses (Ad means adenovirus)**

| Adenovirus profile | NO.OF ISOLATES STRAIN | Percentage |
|---|---|---|
| Ad 3 | 3 | 3.6% |
| Ad4 | 24 | 28.9% |
| Ad8 | 7 | 8.4% |
| Ad11 | 3 | 3.6% |
| Ad19 | 19 | 22.8% |
| Ad37 | 4 | 4.8% |
| Unknown Type | 23 | 27.7 % |
| TOTAL | 83 | |

## 4. Discussion

Conjunctivitis is the inflammation of the clear inside lining of the eyelids and the outer layer of the sclera and is commonly referred to as “pink eye.”

Viral conjunctivitis is an infection of the eye in which one or both eyes become red and uncomfortable. The condition is not normally serious and in most cases clears

up without treatment. It is highly infectious and care needs to be taken to prevent others from becoming infected, for example by not sharing towels .In a small number of cases viral conjunctivitis can lead to the development of small opaque areas within the cornea (the clear window at the front of the eye), which can impact on vision [11].

The procedure of taking a conjunctival swab containing sufficient material for testing requires experience and can be very unpleasant for the patient. Therefore laboratory confirmation of the diagnosis of adenoviral conjunctivitis may not always be carried out. There may be additional reasons for the low number of positive results, for instance, that the samples were taken at a late stage of disease development or that samples were inadequately stored, and the low sensitivity of direct adenoviral immunofluorescence test. The wide age range of the patients' involved in the study (from 3 months up to 46 years) could be another factor.

Ad-CS is more contagious than other forms of conjunctivitis (or even other viral conditions including herpes simplex virus or human immunodeficiency virus) partly due to the virus's ability to remain infectious in the desiccated state for weeks at room temperature. Adenoviruses have no outer lipid bilayer and are highly resistant to disinfection. The virus is transmitted directly through droplets or smears of infected bodily fluids, primarily tears or respiratory secretions, and by fomites on towels, doorknobs, soap, counters, instruments, eye drops, and eyeglasses. The hands of nearly 50% of patients with Ad-CS presenting for care were culture positive. [12]

Saitoh-Inagawa et al [13] and Ishii et al [14] conducted comparative study to investigate viral conjunctivitis in three cities of East Asia: Sapporo (Japan), (Taiwan); and (South Korea). The proportional of adenoviral conjunctivitis in Sapporo, were 52%, 46%and 55%. The common serotypes of subgroup D Ad8, Ad19, and Ad37 were the predominant agents of keratoconjunctivitis, in Israel the most prevalent adenovirus serotypes found were serotypes 1 (22.8%), 2(19.2%), 7 (18%), and 3 (14%) [15,16].

Our study was done in Jeddah (Saudi Arabia) and revealed that adenoviral conjunctivitis constitutes 22.6% of cases presented with follicular conjunctivitis. The predominant serotype was Ad4 24 out of 83 (28.9%), then Ad19 was 19 out of 83 (22.8 %), whereas Ad8, Ad37, Ad11, Ad3 and were identified in 8.4%, 4.8%, 3.6 %, and 3.6% respectively. This warrants that these less common serotypes may lead to an outbreak in the future.

A similar study was conducted between 1999 and 2002 in Egypt [17], using serotype-specific primer sets for PCR amplification rather than common primers followed by sequencing of the PCR product. Therefore, rare serotypes were probably not identified. Nevertheless, the most common serotypes were serotypes 7 (58%) and 3 (16%) of subgroup B and serotype 1 of subgroup C (12%).

Woodland et al in 1992 [18] investigated the causes of acute conjunctivitis and keratoconjuntivitis in 338 patients who attended eye casualty departments in Karachi (Pakistan) during a 5 month period. Most of these infections were diagnosed as adenovirus 291 (75%) or bacterial 71(18.3%) of the remainder, 9cases (2.3%) were caused by herpes simplex virus and 7 (1.8%) by Chlamydia trachomatis. This study highlighted the predominant role of adenovirus as a causative agent of acute conjunctivitis.

In our study 14 cases out of 83 (16.8%) were health care associated infection. In the ophthalmology office, hand washing, furlough for infected employees and proper cleaning of surfaces and instruments have been shown to reduce the risk of viral spread. We postulated the role of cone of applanation tonometer in the spread of infection. The infection control unit had activated a protocol for cleaning and disinfection of these cones using hydrogen peroxide 3%. This has been emphasized by other investigators [19,20] who reported that controlling the spread of adenoviral conjunctivitis in the hospital and the physician's office is of particular importance. The increased risk that adenoviral infection poses to children has already been mentioned. Special care should be taken not to spread adenovirus and other organisms with contaminated instruments.

In conclusion, our study confirmed the endemicity of adenoviruses as etiological agent of acute follicular conjunctivitis and most predominant genotype Ad4&Ad19. To prevent healthcare-associated outbreaks of adenovirus infections, such as epidemic keratoconjunctivitis, health care providers should strictly follow infection control practices, especially hand washing and disinfection of equipment and surfaces and Also follow contact and droplet precautions, and promptly respond to and report clusters of cases.

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[11] Conjunctivitis (viral, non-herpetic) Version 10, Page 2 of 3 Date of search 29.04.14; Date of revision 23.09.14; Date of publication 28.10.14; Date for review 28.04.16 © College of Optometrists.

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