

Frequency of adenoviral conjunctivitis by cell culture and PCR method in two referral university hospitals in Tehran

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ABSTRACT

Background: Ocular adenoviral infections occur throughout the world in both sporadic and epidemic forms. In the present study we determined the frequency of adenoviral conjunctivitis in two referral university hospitals in Tehran by cell culture and PCR method.

Materials and methods: Specimens were scraped from the lower palpebral conjunctiva of 115 patients with conjunctivitis who had referred to Labafinejad and Imam Hossein hospitals during a 6-month period and transferred to two different media, one for cell culture and the other for PCR. Then specimens of cell culture were inoculated to Hep-2 cells and sub cultured in micro plates. Cultures were evaluated for CPE. Viral DNA was extracted from specimens and PCR technique was applied by special primers.

Results: Of 115 samples, 18(15.7%) were adenovirus positive during PCR analysis, of which 14 revealed to be cell culture positive as well. Most of the sufferers from adenoviral conjunctivitis were female (61%) and aged 41-50 years (50%). In patients with adenoviral conjunctivitis, conjunctival hyperemia, pain and eyelids edema were the most common findings.

Conclusion: Results have demonstrated that adenoviruses are common causative agents for viral conjunctivitis. PCR revealed to be more sensitive and accurate than cell culture for detecting adenoviral conjunctivitis.

Keywords: *Adenovirus, Conjunctivitis, Polymerase chain reaction.*

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INTRODUCTION

Conjunctivitis is a common ocular complication seen in general practice and eye clinics. Its etiology includes viral, bacterial, and parasitic infections as well as allergy, trauma, and dietary deficiencies. Among the common microbial causes are Adenoviruses (1). Human adenovirus (HAdVs) belongs to the genus Mastadenovirus of

the adenoviridae family and entails numerous serotypes (2). These viruses infect billions of people worldwide and cause various clinical manifestations including conjunctivitis, upper and lower respiratory tract infections, hemorrhagic cystitis, and gastroenteritis (3).

Ocular adenovirus infections occur in both sporadic and epidemic forms, while large scale outbreaks of epidemic keratoconjunctivitis can occur in hospitals, schools or military establishment. Additionally, failure to diagnose

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ocular adenoviral disease can result in outbreaks of epidemic keratoconjunctivitis (1).

Unfortunately, there are no effective antiadenoviral drugs, thus, prophylaxis is the most important means for preventing adenoviral conjunctivitis (4). Furthermore, prompt and accurate diagnosis of adenovirus is of utmost importance.

Prior investigators have reported a prevalence of 5-10% for adenoviral conjunctivitis (3), however, its epidemiologic profile is unclear in our country, therefore, we determined the frequency of adenoviral conjunctivitis by cell culture and polymerase chain reaction (PCR) in two university hospitals in Tehran.

PATIENTS and METHODS

This descriptive study was conducted during a 6-month period in Shaheed Labafinejad and Imam Hossein hospitals affiliated to Shaheed Beheshti Medical University in Tehran. Totally, 115 patients with conjunctivitis were randomly selected. Acute conjunctivitis (conjunctival hyperemia, discharge, pain and burning) was diagnosed by an experienced ophthalmologist. Having filled the written informed consent, specimens were scraped from the lower palpebral conjunctiva with two cotton swabs and collected in two different transport media (2 ml); one for PCR (distilled water) and the other for cell culture isolation (Hanks balanced salt solution, pH=7.4, FBS=5% and antibiotics).

Virus isolation: Hep-2 cells cultured in 24-well micro plates were infected with the specimens in a volume of 50µl/well and cultured for 1 week. The cultures negative for CPE (cytopathic effect) were subcultured further. Adenovirus-infected cultures showed rounding, ballooning and clustering of cells. Specimens that failed to induce CPE after 3 sub cultures were considered negative.

PCR: For DNA lysis, samples were centrifuged for 30 minutes at 12000xg. DNA was extracted

from the pellet in 98µl of lysis buffer (10 mM Tris-HCl (pH=7.4), 10mM EDTA, 0.5% SDS), and 500µg/ml of proteinase k for 1 hour at 55°C, then the solution was heated for 10 minutes at 95°C. The 1004 bp of the hexon gene was amplified with 50pmol of a pair of primers, AdTU7 (positions 20734 to 20753; 5'-GCCACCTTCTCCCATGGC-3') and AdTU4' (positions 21737 to 21718; 5'-GTAGCGTTGCCGCGCCGAGAA-3').

PCR was carried out in a Mastercycler personal thermal cycler (Eppendorf, Hamburg: Germany), by using a cycle of denaturation at 94°C for 1 min, annealing at 38°C for 1 min, and primer extension at 72°C for 7 minutes. As a positive control in all experiments, 1pg of Ad DNA obtained from cell culture was used per reaction mixture. PCR mixture with DNA of scraping from unaffected eyes was used as negative controls. After the PCR amplification, 5µl of product was subjected to electrophoresis on a 3% agarose gel containing 0.5µg of ethidium bromide per ml. The patterns of the fragment were analyzed by comparison with positive control.

Data analysis was performed by t-test in SPSS soft ware (version 10, SPSS Inc., USA).

RESULTS

The study population included 60 females and 55 males, of whom 11(61.1%) females and 7(38.9%) males revealed to have adenoviral conjunctivitis. Most of the sufferers (50%) aged 41-50 years.

Conjunctival hyperemia (98.2%), purulent secretion (72%), eyelid edema (64.3%), tearing (60%), pain or burning (58.2%), photophobia (29.5%), serous secretion (24.3%), pharyngitis (21.7%), fever (5.2%) and subconjunctival hemorrhage (5.2%) were the most common manifestations of patients with conjunctivitis, however, for patients with adenoviral conjunctivitis clinical manifestations were as follow: conjunctival hyperemia (100%), pain or burning (88.8%), eyelid

edema (55.5%), tearing (55.5%), purulent secretion (55.5%), photophobia (38.8%), pharyngitis (16.6%), fever (16.6%), and serous secretion (16.6%).

Based on PCR technique, 18 samples (15.7%) were adenovirus positive, of which 14 revealed to be cell culture positive as well while 4 samples were positive only for PCR.

DISCUSSION

Globally, the actual prevalence and incidence of adenoviral conjunctivitis and keratoconjunctivitis (EKC) are underestimated since most of the cases are visited by general practitioners and optometrists and are not reported to any medical authority (5). Human adenoviruses (HAdVs) are the major causative agents of EKC and acute conjunctivitis in several societies, especially in East and Southeast Asia (6), however no informative data are available in Iran.

Torres Rojas et al. showed an incidence of 20% (95% confidence interval:14-26%) for adenoviruses in viral conjunctivitis in Cubana (7). This is in agreement with ours. We have found a frequency of 15.7% for adenoviral conjunctivitis among a group of Iranian patients suffering from conjunctivitis.

EKC is endemic in East Asia and other parts of the world. Viruses are isolated from more than 50% of EKC cases, among which more than 94% are adenoviruses (5); however, we have diagnosed only 2 cases of EKC, none of which were adenovirus positive.

Virus isolation by cell culture requires viable organism and experienced laboratory staff. It is costly and time consuming but remains the "gold standard" for infectious agents (1). In the present study, we performed cell culture in micro plates and concluded that this approach was quite sensitive and convenient.

Virus isolation is a time-consuming procedure; therefore, a rapid and reliable technique should be

employed to detect viral agents. PCR has been shown to be more sensitive, accurate and rapid than cell culture for detecting adenovirus (1). Cooper et al compared PCR and cell culture for detecting adenovirus in eye swabs of 415 patients, of whom 386(93%) were positive by PCR compared with 248(59.7%) by cell culture (8). Similarly, we have found that PCR is more sensitive and accurate than cell culture for detecting adenoviral conjunctivitis.

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