

Original Article

Incidence of Adenoviral Conjunctivitis in Patients Referred to the Iran University Affiliated Hospital

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Abstract

Background and Aims: Adenovirus is one of the causative agents of viral conjunctivitis. Approximately an average of 40% of viral conjunctivitis is due to adenovirus infection. The rate of infection is usually the highest during the spring and summer months. In this study attempt was made to evaluate the incidence of conjunctivitis due to adenovirus infection in patients referred to one of the affiliated university hospital using the congenital virological methods.

Materials and Methods: Samples were taken by a swab from patients with clinical conjunctivitis. Samples were processed and tested using the techniques of cell culture inoculation and polymerase chain reaction.

Results: From the 100 samples taken 16% of them were positive by PCR Method. From these only 8% showed viral growth on cell culture. There was no difference of infection between the sex groups but most cases accrued in patients aged 17-27 years during the months of March to May.

Conclusion: From the results of this study it was concluded that adenovirus plays a major role as a causative agent of conjunctivitis.

Keywords: Infectious bronchitis virus (IBV); Avian influenza virus (H9N2); Massachusetts; 793/B serotype; multiplex RT-PCR

Introduction

Adenovirus serotypes represents the most common pathogenic cause for a red eye worldwide (1). The prevalence of adenoviral conjunctivitis was found to represent between 15% and 70% of all conjunctivitis cases in the worldwide both in sporadic and epidemic forms, and large scale outbreaks of epidemic keratoconjunctivitis can occur in hospitals, schools, military establishments (2-11). Human adenovirus are classified into 6 subgenera and 51 serotypes

(12). Approximately one third of the human adenovirus serotypes have been associated with common forms of adenoviral-related eye infections (12), but the most common causes of acute conjunctivitis are related to serotypes 3,4,8,11,19,and 37 (13).

Clinically, adenovirus infections are diagnosed on the basis of history, symptoms, and signs (13). However, it sometimes can be difficult to distinguish bacterial from viral conjunctivitis (14).

The conventional technique for diagnosis of viral conjunctivitis includes conjunctival cytologic investigation in which inoculation of susceptible cell lines with specimen taken from an infected eye are followed by the observation of cytopathic effect (15).

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Although this technique is costly and time-consuming but remains the gold standard because isolation of an infectious agent is definitive (16).

More recently, Since two decades ago, the technique of polymerase chain reaction (PCR) has been used widely for the diagnosis of adenovirus infection in clinical ophthalmology (17).

The technique has been shown to be more sensitive, accurate, and less time consuming than cell culture for detecting adenovirus from cases of conjunctivitis (18-20).

Detection of adenovirus by cell culture procedure is performed rarely for conjunctivitis because of the considerable time delay in results and the self-limited nature of typical conjunctivitis (21). Because of ocular adenoviral infections occur worldwide in both sporadic and epidemic forms, it was decided to evaluate the incidence of adenoviral conjunctivitis by using the combined techniques of cell culture and PCR on specimen taken from patients referred to the ophthalmology department at Rasoul Akram Hospital.

Methods

Collection of specimen

patients with clinical signs of sore eye and conjunctivitis attended the ophthalmology clinic at Rasoul Akram Hospital, Iran University of Medical sciences during 6 Month period from January to the first of July, 2007. Patients were examined by a specialist and two specimen were taken from each patient using two sterile Dacron swabs. The swabs were placed separately into two sterile polystyrene tubes each containing 1ml of transport Medium (DMEM).

The tubes were kept at 4°C and delivered on cold bag to the lab where they were kept frozen at -70°C till used. A total of 100 patients were sampled.

Cell culture

HeLa cells were cultivated as monolayer in disposable culture flasks containing Dulbecco Minimal Essential Medium (DMEM) with antibiotics and 5% fetal calf serum. The flasks

were incubated at 37°C in an atmosphere containing 5% CO₂. For comparison, Vero cells were also grown similarly and used for virus isolation.

Virus isolation

HeLa cells were grown as monolayers in 24 wells tissue culture microplates. The cells were maintained in DMEM supplemented with 5% FCS and incubated at 37°C in an atmosphere containing 5% CO₂.

Clinical specimen in transport Media were centrifuged in a microfuge for 2 minutes. The supernatant was saved and 100µlit of it was inoculated into each well of cell monolayer and allowed to adsorb for 1 hr at 37°C. The inoculated cells were kept under the above condition and examined daily for the appearance of CPE.

PCR

DNA was extracted from the clinical specimen using phenol chlorom extraction method.

The primers had been designed from the hexon gene of adenovirus and their sequences were as Follow:

ADRJC1(5-GACATGACTTTCGAGG
TCGATCCCATGGA3)

ADRJC2 (3-ATGG ACGCGTGGGGA
AGAGT CG GCC-5).

Thermal cycler was programmed for one initial cycle of 94°C for 1min, 55°C for 1min and 72°C for 1min Followed by 40 cycles each at 94°C, 1min, 55°C for 1min and 72°C for 1.5min. The PCR products were electrophoresed on 2% agarose gel containing Ethidium bromide. The bands were visualized using an ultraviolet Trans illuminator.

Data analysis was performed by chi-square and T-Test in SPSS software (version16).

Results

During the 6-month period from the beginning of January to the first of July 2009-2010, 100 samples were taken from the eyes of patients with possible viral conjunctivitis. The study population consisted of 45 females and 55 male. The minimum age of the patients was 2

Table 1: Frequency of adenovirus positive cases based on sex.

total		Infectious agent				Age-group
		others		adenovirus		
%	Total pos	%	pos	%	pos	
2	2	2.4	2	0	0	<5
6	6	6	5	6.2	1	6-16
39	39	34.5	29	62.5	10	17-27
16	16	17.9	15	6.2	1	28-38
17	17	17.9	15	12.5	2	39-49
11	11	13.1	11	0	0	50-60
4	4	3.6	3	6.2	1	61-71
5	5	4.8	4	6.2	1	72<

years and maximum age was 77 years (Table 1), with the mean average age of 29.25. The clinical symptoms of patients are shown in table 3.

PCR Assay

DNA from all the specimen was extracted and subjected to PCR assay using adenovirus specific primers. From the total of 100 specimens tested 16 were positive by PCR test. The positive specimen showed a DNA band with a size equivalent to 150 bases (Fig. 2).

Discussion

Although conjunctivitis typically is caused by viruses, bacteria, or allergy, adenovirus is the most common cause of infection (22). Adenovirus represents 15 % to 70% of all worldwide cases of infectious conjunctivitis (23).

Tress Rojas *et al*, showed an incidence of 20% for adenovirus in viral conjunctivitis in Cuban (24) which is similar to our results . We have found a frequency of 16% for adenoviral conjunctivitis among Iranian

Table 3: Distribution and frequency of signs in patients.

total		sex				Infectious agent
		female		male		
%	Total pos	%	pos	%	pos	
16	16	22.2	10	10.9	6	Adenovirus
84	84	77.8	35	89.1	49	Other cases
100	100	100	45	100	55	total

Table 2: Frequency of cases based on age and infectious agent.

sign	And% of patient in whom present
Hyperemia	87.5
Pain	87.5
Tearing	87.5
Itching &irritation	75
Edema	68.8
Follicle	31.2
pharyngitis	6.2

patients, whereas Kasparo *et al*. showed an incidence of 61% for adenoviral conjunctivitis (25). The difference could indicate geographical and hygienic discrepancies between different zones of the world.

The results of this study showed that the incidence of adenoviral conjunctivitis were the most in March and May. Adenoviral conjunctivitis demonstrates seasonal variability, it is often higher during the summer and is thought to be related to the increased risk of transfer seen with summer-related activities such as swimming and day camps (26).

Therefore, it seems incidence of this disease will increase during summer.

Statistical analysis have shown that there is no significant correlation between gender and disease. But there is significant correlation between age and disease and also contacting with infected people. So close contact with people can influence transmission of disease.

For detecting conjunctivitis due to adenovirus infection the techniques of cell culture and PCR were used. Regarding the results in this study PCR has been shown to be more sensitive, accurate than cell culture. Cooper *et al*. In1999 compared two technique PCR and cell culture for detecting adenovirus and the results showed that PCR is more sensitive, accurate and rapid than cell culture .Since isolation of an infectious agent is definitive, cell culture remains the gold standard (27).

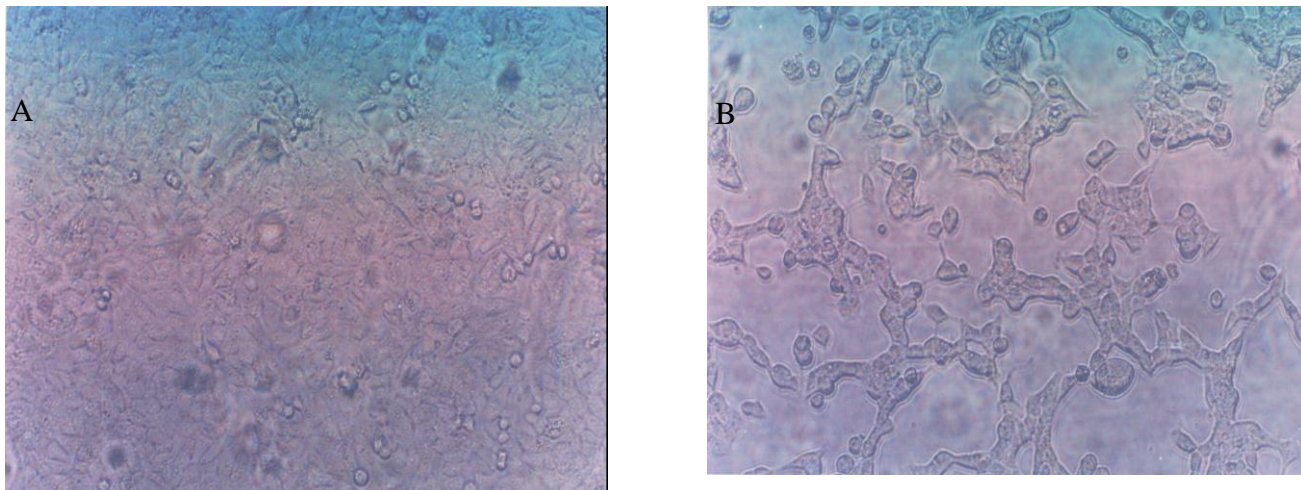


Fig. 1: A) Normal HeLa cells. B) CPE in the HeLa cells at 2 days after infection with adenovirus type 5.

On the other hand, we know both of them are time - consuming and expensive. Therefore, regarding the incidence of adenoviral conjunctivitis, to decrease the spread of disease and limit the toxicity, allergy and antibiotic resistance associated with unnecessary treatment. The need for a rapid and reliable technique for detecting adenoviral conjunctivitis seems necessary.

Recently, the US Food and Drug Administration cleared a diagnostic test for detecting adenoviral conjunctivitis, the RPS (Rapid Pathogen Screening) adeno detector (22). A multicenter clinical trial found a sensitivity of 89% and specificity of 94% for

We suggest the test to be used in order to prevent unnecessary antibiotic treatment.

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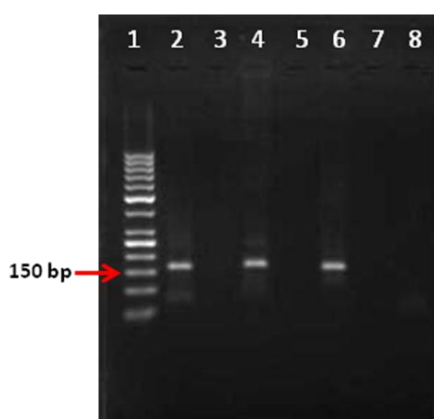


Fig. 2: lane 1: Ladder 50 bp, Lane 2: Positive control, Lane 3: Negative control, Lane 4, 6: Positive samples, Lane 5, 7, 8: Negative samples.

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