# The Prevalence of Viral Conjunctivitis in Patients Who Referred to Eye Specialist Hospitals in Tehran, Iran

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**Abstract**

***Purpose*:** The main purpose of this study was to evaluate the prevalence of herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Varicella-Zoster virus and Adenovirus associated with conjunctivitis in swab samples of patients who referred to Eye Specialist Hospitals in Tehran.

***Methods*:** In this cross-sectional study, swab samples from patients with acute conjunctivitis during the first sixth months of 2012, in two ophthalmic departments of Tehran were collected. After DNA extraction, multiplex Real-Time PCR was carried out in two separate reactions for each sample.

***Results*:** A total of 150 swap samples were collected from acute conjunctivitis patients. Among them, 22 (14.6%) and 5 (3.3%) were positive for Adenovirus and HSV-1 DNA, respectively. There were no patients with positive results for HSV-2, VZV. Statistical analysis showed insignificant P-value (0.845) between gender and conjunctivitis; however, there was close correlation (P-value $<0.05$) with age. Moreover, 63.6% of Adenoviral conjunctivitis occurs in the summer compared to spring (36.4), whereas no significant seasonal variation was observed for HSV-1.

***Conclusion*:** This prevalence pattern indicates that adenovirus has major role in viral conjunctivitis rather than HSV-1. Further research is needed to identify other viral pathogens associated with ocular infection.

***Keywords*:** Adenovirus, Conjunctivitis, Herpes Simplex Virus, Multiplex Real-Time PCR

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## Introduction

Conjunctivitis is one of the most common of ocular morbidity in the world. Microbial agents as well as allergy and trauma are the most frequent etiological factors for conjunctivitis.[1,2] Viral conjunctivitis in their early stages can not be differentiated clinically from each other and also from bacterial or allergic conjunctivitis. Among the viral agents, Human Adenoviruses (HAdV), herpes simplex 1, 2 (HSV-1, HSV-2), Varicella Zoster Virus (VZV) are the important recognized causes of this disease.[2,3]

Both sporadic and epidemic outbreak forms of Adenoviral conjunctivitis occur in all age groups throughout the world.[4-6] Highly epidemic form, acute hemorrhagic conjunctivitis are often spread in family, hospitals, schools, military establishments and office.[7]

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HSV is endemic in every human society and conjunctivitis caused by this virus is potentially blinding.[8]

Eye infection caused by VZV usually when it become reactivated in the ophthalmic division of the trigeminal nerve. In this condition, a minority of patients may develop conjunctivitis, keratitis, uveitis, and ocular cranial-nerve palsies.[9]

There are various methods for the laboratory diagnosis of viral infections, such as viral culture, antigen detection, serology, and nucleic acid detection. The latter is more sensitive and is not dependent on the presence of viable virus or the quality and presence of appropriately infected cells.[10] Therefore, polymerase chain reaction (PCR) is now emerging as the "gold standard" for the diagnosis of viral conjunctivitis.[9,10] Traditional PCR is dependent on electrophoresis of the PCR product and analyzed them in the presence of ethidium bromide by ultraviolet light.

In the recent years, introduction of real time PCR has markedly increased the speed in the virology laboratory due to the relevant technology that permits rapid temperature cycling within a closed system.[11] Real-time PCR has provided a great deal of accuracy, short turn around time, and low potential for carry-over contamination in comparison with conventional PCR methods.[12] As uniplex real time PCR has been established as a sensitive technique to only recognize one specific virus at the time, the aim of this study was to use of the HSV, Adenovirus and VZV multiplex real-time PCR assay as an easy and sensitive method to evaluate the prevalence of HSV-1, 2, VZV and Adenovirus in swab specimens from patients with conjunctivitis who referred to Eye Specialist Hospitals in Tehran

## Methods

In this cross sectional study, conjunctival swab was obtained from symptomatic patients with signs of conjunctivitis who had referred to Farabi and Labbafinejad hospitals during spring and summer of 2012. conjunctival swabs were placed in 1 ml of Viral Transport Media (VTM). Specimens were stored at -70°C until time of processing.

Viral DNA was extracted from 200 µL of clinical samples using DNA-QIAamp Kit (QIAGEN Inc., Valencia, CA) according to the instruction of the manufacturer. Extracted DNA was eluted in final volume of 50 µL of supplied elution buffer and stored at -20˚C until tested.

For diagnostic assay, Multiplex TaqMan Real-Time PCR Kit (FTD eye, Fast-track Diagnostics Luxembourg S.a.r.l) was used. Two tube multiplex Real-Time PCR reactions for detection of HSV-1, 2, varicella-zoster virus and adenovirus, including internal control (IC), was done. Reactions were set-up and performed according to manufacturer's instructions. The 15 µL PCR reactions contained of 7.5 µl Premix Ex Taq™ (Perfect Real Time) (Takara, Bio Inc., Shiga, Japan), 2.5 µl Primer/probe mixer and 5 µl of purified DNA. Reactions were performed in a Rotor-Gene 6000 with the following parameters: initial template denaturation at 95°C for 30s followed by 40 cycles, each consisting of 95°C for 5s and 60°C for 34s. The presence of specific viral sequences in the reaction was indicated by an increase in the fluorescence from the relevant dual-labeled probes, and was reported as a cycle threshold value (Ct) by the real time thermocycler. Data analysis was performed using Rotor-Gene 6000 software. In order to discern PCR inhibitors which are responsible for false-negative results, we added to samples murine cytomegalovirus (mCMV) as an IC that co-amplified with the targets DNA. It was introduced into the lysis buffer at the extraction stage of each sample and the positive and negative control. All runs included negative and positive control.

In this study, Pearson $\chi^2$ and Spearman's rho were used for Statistical analysis. A p-value of $<0.05$ was considered to be statistically significant.

## Results

During the period of study, a total of 150 patients with signs of conjunctivitis were referred to Farabi and Labbafinejad hospitals. The patient's age range was from two to 80 years with a mean age of 29.00±10.68 years. There were 51 (34%) females and 99 (66%) males in this study. The prevalence of HSV-1 and 2, Varicella- Zoster virus and Adenovirus was assessed in these patients. In all of the patients who were enrolled in this study, 22 (14.6%) patients with Adenovirus DNA and five (3.3%) with HSV-1, there were no patients

with positive results for HSV-2 and VZV. Furthermore, Adenovirus was more frequently distinguished during the summer compared to spring, whereas no significant difference was observed in distribution of HSV-1 in spring and summer (Figure 1).

Men had a higher burden of conjunctivitis than women; however this difference was not statistically significant (p=0.845). Furthermore, statistical analysis showed close correlation between age and conjunctivitis which was statistically significant ($p<0.05$). A high proportion of conjunctivitis swaps were negative for expected viruses in this study (82%).

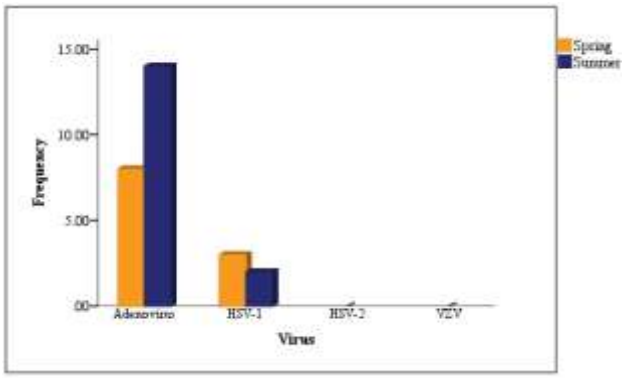


**Figure 1.** 63.6% (14 of 22) adenoviral cases were seen at summer. Seasonal fluctuation was not observed for HSV-1

## Discussion

In the present study, Multiplex Real-Time PCR was performed to evaluate the prevalence of HSV-1, 2, VZV and Adenovirus in a total of 150 patients with signs of conjunctivits that were referred to Farabi and Labbafinejad hospitals during spring and summer of 2012. In all of the patients, 22 (14.6%) patients with Adenovirus DNA and 5 (3.3%) with HSV-1, there were no patients with positive results for HSV-2 and VZV.

Generally, patients with ocular viral diseases are given treatment based only upon clinical appearance by ophthalmologists; thus the true prevalence of all ocular viral diseases might be unclear. Certainly, it would be valuable to report etiological diagnosis of viral diseases and predict the value of them. Therefore, it is crucial that a rapid, sensitive and specific diagnostic method be exploited to prevent from complications that can be a consequence of misdiagnosis.

Infective conjunctivitis is a highly contagious eye disease that causes chiefly by Adenoviruses which has worldwide distribution.[4] Adenoviruses are representing 15 to 70% of all conjunctivitis cases globally.[13,14] Adriana et al revealed 60% frequency of adenoviral conjunctivitis in the population of Rio de Janeiro, Brazil, between March 2004 and May 2007.[15] Moreover, Yagci et al reported 26.5% (9 of 34) by the PCR method in Turkey.[16] In this study of 150 conjunctivitis cases (%14.6) 22 samples were positive for adenovirus by Real-Time PCR. Our data is similar to the results which are obtained from two researches in the past years but with different methods. They reported adenoviral incidence 16% and 15.7% respectively in Tehran, Iran.[17,18] As a whole, similarity of the results shows that within several years, a significant change in the epidemiological pattern of disease has not occurred in the referral centers.

Epidemiological studies reported relationship between HSV and ocular disease. In 1978, Neumann-Haefelin found in a continuous series of 457 patients that 154 had HSV-1 and 3 had HSV-2 (1.9%).[19] According to our assay, the incidence of HSV-1 at ocular infection was 3.3%. HSV-2 and VZV, by contrast, had no positive samples. This difference could be caused by geographical and sanitary conflicts between different regions of the world.

In Iran, little research has been done in this field; because ocular viral diseases are given treatment based on clinical appearance.

In this study, statistical analyzes have shown that there is no significant relationship between gender and disease. Nevertheless, the incidence and prevalence are influenced by age. This factor may affect the degree of exposure to these sources of infection. In addition, we conclude that Adenovirus was more common during summer compared to spring in spite of the fact that no seasonal fluctuation was observed for HSV-1. In Japan, adenovirus was also detected most frequently in the summer.[20]

However, due to the restricted reliability of clinical diagnosis of adenovirus, HSV, and VZV, powerful techniques for detection of these agents in conjunctival swabs is often precious and preventing misdiagnosis of ocular adenoviral disease and plays pivotal

role in dealing with outbreaks of epidemic keratoconjunctivitis. The ideal test for diagnosing ocular pathogens has been defined as one whose result be available before the patient leaves the doctor's office.[21] Due to inherent sensitivity, high specificity and rapid result, Real-Time PCR is considered as the ultimate modern diagnostic tool for the identification of adenovirus, HSV and other viral agents. Moreover, screening of microbial infection by Multiplex Real-time PCR not only would allow rapid detection of a variety of pathogens simultaneously, which will in turn help to the treatment and clinical management of the patient,[22] but also aid investigator to determine the epidemiological patterns as a professional strategy. However, in the current economic conditions, disadvantage of this method is its high cost that could not be performed routinely to diagnose eye infection. At the end it should be noted that in this project, all of the enrolled patients were suffering from conjunctivitis but only 18% of them were infected with adenovirus or herpes. As a result, the role of other vial factors such as picornaviruses remains to be unknown.

## Conclusion

In conclusion, we recognized that adenovirus is the most important causes of conjunctivitis among the investigated viral agents. And the other causes of conjunctivitis in Tehran have not been included in this report. Therefore subsequent research is needed to identify other microbial factors associated with ocular infection.

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