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# Molecular Prediction for the common causative agents of infectious conjunctivitis among AL-Najaf AL-Ashraf governorate patients in spring and summer.

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## Abstract

**Aim of the study**: is to predict the most common causative agents of infectious conjunctivitis among AL-Najaf AL-Ashraf governorate during spring and summer seasons.

**Methods** : Multiplex PCR was used to amplify viral and bacterial genes related to pathogens suspected of causing infectious conjunctivitis, the samples were collected from patients using cotton eye swabs.

**Results**: A total of 36 (10.29%) patients were chosen from 350 patients who attended private ophthalmic clinic in AL-Najaf AL-Ashraf governorate during spring and summer seasons. Two Cotton swabs were taken from each patient and kept frozen until the day of DNA extraction. Multiplex PCR was used to amplify genes specific for Adenovirus, Herpes Simplex Virus-1 and Herpes Simplex Virus-2, Cytomegalovirus, Varicella Zoster Virus and *Chlamydia trachomitis* plasmid. The multiplex PCR results found that only ten (27.77%) samples were positive for mixed infection of Adenovirus (140bp), HSV-1and HSV DNA polymerase (208, 211 bp) and only one (2.77%) sample was positive for HSV-1mixed with HSV DNA polymerase .

**Conclusion**: Laboratory identification of infectious agents causing conjunctivitis is crucial for confirming the clinical diagnosis, although some signs may be diagnostic for certain types of eye disorders because infectious and allergic conjunctivitis signs and symptoms may overlap.

Key words: conjunctivitis, allergic conjunctivitis, adenovirus, herpes simplex virus.

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## Introduction

Conjunctivitis is a common term for inflammation of the conjunctiva, that might be acute, chronic, or recurrent. Conjunctivitis can be classified into two different types, the infectious conjunctivitis which is caused by different microbial agents like bacteria, viruses, fungi and parasites, and the noninfectious conjunctivitis which is caused by noninfectious causes like allergic, chemical, mechanical, toxic, immune-mediated, and neoplastic [1].

One of the commonest conjunctivitis type is the viral conjunctivitis, it is characterized as contagious infection with several symptoms such as eye irritation, excessive tearing, soreness, foreign body sensations, light sensitivity and blurred vision in advanced cases. Ocular disorders include blepharedema, epiphora, conjunctival hyperemia and others. Adenoviruses being one of the most common causes of viral conjunctivitis followed by Herpesviruses, including Herpes simplex virus and Varicella-zoster virus, which also causes chickenpox and shingles [2,3].

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Bacterial conjunctivitis is commonly present as an acute form of infection. The disease is commonly unilateral with hyperemia and purulent discharge, among sexually active people via hand to eye can cause chronic follicular conjunctivitis in adults/ neonate by *C. trachomatis* serotypes D-K [3,4]. Type I allergy consider the main sensitivity types manifest the Allergic conjunctivitis[5].

The recommended treatment for conjunctivitis depend mostly on whether it's caused by infection, an irritant or an allergic reaction. So, connecting type of conjunctivitis to its cause and season of occurrence will aid in treating patients and preventing recurrence of such diseases. Thus, the current study was conducted to predict the common causes of conjunctivitis among AL-Najaf AL-Ashraf governorate patients during spring and summer seasons.

## Materials and methods

**Study group and specimens:** the study was started on March to October 2017, a total of 36 (10.29%) patients were chosen from 350 patients who attended private ophthalmic clinic in AL-Najaf AL-Ashraf governorate during spring and summer seasons. Two Cotton swabs were taken from each patient and kept frozen until the day of DNA extraction. The specimens were swabbed from the lower palpebral conjunctiva with two cotton swabs.

### Inclusion criteria

All patients with typical signs and symptoms of acute infectious conjunctivitis were included in the current study.

### Exclusion criteria:

all patient with / or suspected to have history of allergic or any type of conjunctivitis other than infectious conjunctivitis.

### A. DNA extraction

KAPA Express Extract Kit (KR0383 – v4.16, USA) was used to extract the DNA from the swabs according to the followings:

1. Reaction solution Setup

Each swab was placed directly in 300 µL reaction mixture with 0.5X KAPA Express Extract Buffer (15 µL Express Extract Buffer, 2 µL KAPA Express Extract Enzyme, and 283 µL PCR-grade water).

2. Lysis

The lysis was performed in a water bath using the following protocol:

**Cell Lysis procedure for DNA extraction using water bath**

| Step | Water bath Temperature | Duration |
|---|---|---|
| Lysis | 75°C | 10 min |
| Enzyme activation | 95°C | 5 min |

### 3. Dilution

Samples were centrifuged briefly to pellet the cellular debris, the DNA extract was diluted to 10-fold with 10 mM Tris-HCl (pH 8.0–8.5). Extracts were stored at -20°C till the thermocycling day.

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**B. PCR Primer used in the study.**

Seven primers were choose from literature according to the entire DNA sequence of the glycoprotein D (K02372) of HSV-1 and HSV-2, of CMV (M14709), and of VZV (X04370, M14891, M16612) [6], Adenovirus hexon , HSV DNA and *Chlamydia trachomitis* plasmid [7] (Table 1).

**Table (1) Oligonucleotides sequences of the Primers used in the study**

| Primer code | Composition of the primers | Size of product(bp) |
|---|---|---|
| **1. Adenovirus hexon** | 5'GACATGACTTTCGAGGTCGATCCCATGGA3'<br>3'CCGGCTGAGAAGGGTGTGCGCAGGTA5' | **140** |
| **2. Herpes simplex -1 (HSV-1)** | 5'GTTAGGGAGTTGTTCGGTCATAAGCT3'<br>3'TCGGCCATCTTGAGCATCC5' | **208** |
| **3. Herpes simplex -2 (HSV-2)** | 5'GTCGGTGTGGTGTTCGGTCATAAGCT3'<br>3'GGCTGAATCTGGTAAACACGCTTC5' | **276** |
| **4. Herpes simplex (HSV) DNA polymerase** | 5'GACTACCTGGAGATCGAG3'<br>3'TGAACCCGTACACCGAGT5' | **211** |
| **5. Cytomegalovirus** | 5'CACGGCCGCCACCAAGGT3'<br>3'AGTGGTTGGGCAGGATAAA5' | **392** |
| **6. Varicella-zoster virus** | 5'ATCGCGGCTTGTTGTTTGTCTAAT3'<br>3'GGGCGAAATGTAGGATATAAAGGA5' | **355** |
| **7. *C.trachomatis* plasmid** | 5'TCCGGAGCGAGTTACGAAGA3'<br>3'AATCAATGCCCGGGATTGGT5' | **241** |

The reaction of multiplex PCR was performed according to the manufacturer instructions and the thermocycling condition was performed according to the following program: Initial Denaturation : 3 minutes at 95 °C, Denaturation: 15 seconds at 95 °C, Annealing : 30 seconds at 60 °C, Extension : 50 seconds at 72°C, no. of cycles: 30, final Extension: 5 minutes at 72°C. Gel electrophoresis performed on 1.5 gel electrophoresis using TAE buffer and the results were visualized using gel documentation system.

**Statistical analysis**

Percentage and Chi square test were used as a statistic in this study and the P value > 0.05 was considered as a non-significant.

**Results**

**1- Patients History and Physical Examination**

According to signs and symptoms and careful physical examination of 350 patients complaining of different eye disorders there were only thirty six patients of them having infectious conjunctivitis. The patient history showed that eight (22.22%) patients were complaining of flu and only one (2.77%) had pharyngitis in addition to conjunctivitis. Seven patients (10.44%) have family members with ocular disease too. Among female patients only one was pregnant (2.77%) and two women with abortion (5.55%) (Table 2).

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**Table (2) Patients History and Physical Examination .**

| Objective | Number | % |
|---|---|---|
| 1. Total no. of Patients with Conjunctivitis | 36 | 100 |
| 2. Conjunctivitis accompanied diseases | | |
| - Flu | 8 | 22.22 |
| - Pharyngitis | 1 | 2.77 |
| 3. Patients with family history of ocular disease | 7 | 19.44 |
| 4. Pregnancy | 1 | 2.77 |
| 5. History of abortion | 2 | 5.55 |

**2- Laboratory investigation results:**

A total of 10(27.77%) samples appeared positive for Adenovirus hexon (140bp), and for HSV-1and HSV DNA polymerase (208, 211 bp). Only one case (case number 6) (2.77%) appeared positive for HSV-1mixed with HSV DNA polymerase. Twenty five samples (69,44%) were Negative, the results showed a non-significant difference with a P value=0.22399118. (Table 3) (Figures 1,2).

**Table (3) Multiplex PCR results.**

| Positive samples NO. | Adenovirus hexon | HSV-1 plus HSV DNA polymerase |
|---|---|---|
| **2** | **Positive** | **Positive** |
| **3** | **Positive** | **Positive** |
| **5** | **Positive** | **Positive** |
| **6** | **Negative** | **Positive** |
| **9** | **Negative** | **Positive** |
| **10** | **Positive** | **Negative** |
| **11** | **Positive** | **Negative** |
| **12** | **Positive** | **Positive** |
| **21** | **Positive** | **Negative** |
| **23** | **Positive** | **Negative** |
| **24** | **Positive** | **Negative** |
| **Total =11(30.56%)** | P value = 0.22399118 | |

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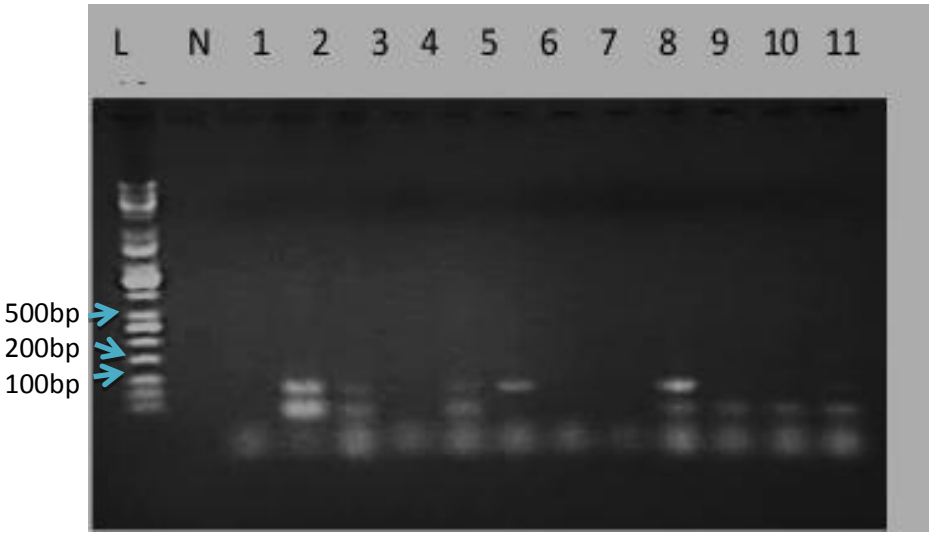


**Figure (1): Multiplex PCR results on 1.5 gel electrophoresis for different microbial primers. The results were positive for Adenovirus Hexon (140 bp) and HSV-1 (208 bp) mixed with HSV DNA polymerase (211 bp) in {2,3,5,6,9,12) , positive results for Adenovirus Hexon (140 bp) only in {10, 11}. L: ladder(BenchTop 100bp Promega DNA Ladder/ USA), N: negative control.**

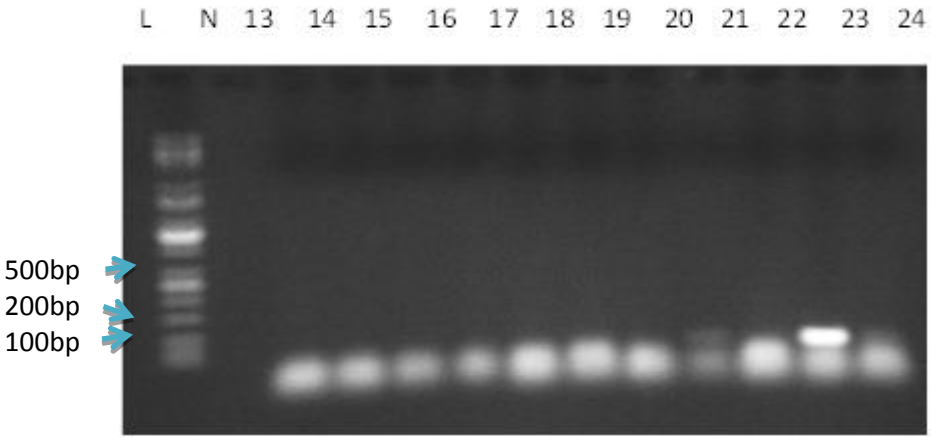


**Figure (2) : Multiplex PCR results on 1.5 gel electrophoresis for different microbial primers. Positive results were showed for Adenovirus Hexon (140 bp) only in { 21,23,24}. L: ladder(BenchTop 100bp Promega DNA Ladder/ USA), N: negative control.**

The association between the patient's history and the positive results revealed that, 3 patients with flu signs, one patient with pharyngitis and one patient with family history of ocular disease appeared positive for Adenovirus with mixed HSV-1 and HSV DNA polymerase (Table 5).

**Table (4) Distribution of Underlying Diseases among Positive and Negative Cases**

| PCR results | Flu No. (%) | Pharyngitis No. (%) | Family history of conjunctivitis |
|---|---|---|---|
| Positive | 3 (37.5) | 0 | 1 (14.28) |
| Negative | 5 (62.5) | 1 (100) | 6 (85.71) |
| Total | 8 | 1 | 7 |

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## Discussion

Conjunctivitis is the most frequent ocular disorder observed in ophthalmic clinics [2]. In our study we tried to detect the causative agents of conjunctivitis in a specific seasons of the year (summer and spring). Primers of seven genomes of the most common causes of viral conjunctivitis and *Chlamydia trachomatis* plasmid primer were amplified using Multiplex PCR technique (Table 1). Some of the patients have other underlying disease other than ocular conjunctivitis like flu (22.22%) , pharyngitis (2.77%). On the other hand, some patients have a family history with ocular disease (19.44%). Ahmed on 2008 said that the viral conjunctivitis is epidemic and contagious. Particularly, Adenovirus conjunctivitis is extremely contagious and can be transmitted between people via inanimate objects. Also others said that the viral conjunctivitis can be transmitted via direct contact with contaminated fingers, medical tools, swimming pool water and personal items [8,9]. However , in our study and according to the results of multiplex PCR results there were only 30.56 % of the cases were positive for the detected infectious agents. Our results is unexpected comparing to the patients clinical signs which confirmed that these cases might be infectious conjunctivitis. The results can be due to an infectious agents other than those we used in our study. Or the studied cases were actually allergic rather than infectious form of conjunctivitis. In our study we used Multiplex PCR which is an effective diagnostic method it is very reliable, simple, rapid and sensitive technique for detecting infectious agents even in low number [6,10].

The current results showed that 10 (27.77%) cases were positive, for adenovirus and HSV-1 mixed with HSV DNA polymerase while one case (2.77%) for HSV-1 mixed with HSV DNA polymerase . Our results agree with the results of Amir who mentioned that Adenovirus can cause around 65% - 90% of viral conjunctivitis [11]. In addition to the National Health and Nutrition Examination Survey which recorded a recent decline in the prevalence of HSV1 and HSV2 (53.9% and 15.7% respectively) [12].

The study found that 69.44% of the samples showed negative results for the mentioned pathogen these cases might be caused other pathogens or it is an allergic conjunctivitis cases[1].

The pink eye occurs seasonally as an allergic conjunctivitis, the presence of grass pollens and dust have been reported to have a relationship with increased ocular symptoms during spring or summer seasons [13]. Our results disagree with Orly 2012 who said that the bacterial is a common cause of conjunctivitis in spring and summer [14]. Bonini in 2009 said that the seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) account for 95% of the allergic eye diseases in the practice. These forms involve both eyes and occur seasonally (spring, summer) [15].

Although, our study depended on careful physical and clinical examination of patients as an inclusion criteria and all cases suspected to have allergy were excluded from the study there are still negative cases. The accurate rate in diagnosing viral conjunctivitis clinically is less than 50% when compared with the laboratory approval. As well as, many cases are misdiagnosed as bacterial conjunctivitis [1,16]. Patients family history of having one or two family member complaining of pink eye also not enough because allergy can be genetic disorder. Another researchers suggest that , Although the eye allergy genetics has been ignored by ophthalmologists, it is still needs to more confirmation [17].

Our study showed that only one (14.28%) patient with positive infectious conjunctivitis have a relative while 6(85.71%) were negative. (Table 5)Seasonal occurrence, may be a helpful criteria in diagnosis, bacterial conjunctivitis affects mostly during December to April, viral conjunctivitis appeared in the summer, whereas allergic conjunctivitis frequently seen during the spring and summer [18].

However, still laboratory investigation along with Clinical diagnosis is the best choice for confirming the type of conjunctivitis. According to the recent Japanese guideline for definitive

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diagnosis of allergic conjunctivitis should rely on clinical diagnosis, positive results for serum antigen specific IgE antibody or skin reaction with presumed antigens and a positive result for eosinophils in the conjunctival smear[5]. In conclusion, Accurate Diagnosis of different types of conjunctivitis is often difficult, but laboratory investigations are important in determining the exact cause. Almost all types of conjunctivitis starts with the same clinical signs [1]. The availability of appropriate treatment for adenovirus and HSV together with the potentially serious residual morbidity of these infections clearly justifies the need for accurate laboratory investigation in cases of infectious conjunctivitis [19]. Allergic conjunctivitis is more common in spring and summer seasons than infectious type [20].

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**Authors' Disclosures of Potential Conflicts of Interest**

No potential conflicts of interest relevant to this article were reported.

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