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Molecular Study of Adenovirus Associated with eyes infections in Iraq

Basim A. H. Jarullah[1], Noor K. Al-Abadi[*2] Sada G. Al- Musawi[2,3], Heekmat K. Ateia[1]
[1] College of Veterinary Medicine, University of Thi-Qar, Thi- Qar, 64001, Iraq.
[2]College of Health and Medical Technologies, National University for Science and Technology, Thi-Qar, 64001, Iraq.
[3] College of Dentistry, University of Thi-Qar, Thi-Qar, 64001, Iraq.
*Corresponding Author Email: noor.abed@nust.edu.iq

Abstract
The current study included the examination of 180 samples of eye swabs for ophthalmitis patients of all ages, and their association with some demographic factors, including age, gender, clinical symptoms, recurrence of infection and chronic diseases by means of viral transmission (VTM), which included 120 samples of patients and 60 normal samples in Iraq From October 2020 until January 2021 . Samples were collected from patients from a consultant ophthalmologist. The samples were classified into five groups according to the age group (1-15), (16-30), (31-45), (46-60) and (over 60) years old. Current study involved collection and storing samples at -20°C, extracting DNA from eye samples using the Viral DNA Kit in accordance with the manufacturer's instructions, analyzing the samples using PCR technology, and performing an electrophoresis to analyze the results of the PCR reaction and separate the DNA from the agarose and depending on the size of the DNA. Adenoviruses were discovered using the PCR method, and the analysis' findings indicated that there were no adenoviruses.
Keywords: adenovirus, Eye infections, PCR

الخلاصة
تضمنت الدراسة الحالية فحص 180 عينة من مسحات العين لمرضى التهاب العين من جميع الأعمار، وارتباطها ببعض العوامل الديموغرافية، بما في ذلك العمر والجنس والأعراض السريرية وتكرار العدوى والأمراض المزمنة عن طريق جمعها في وسط ناقل فيروسي، والتي شملت 120 عينة من المرضى و 60 عينة طبيعية في العراق من تشرين الأول 2020 حتى كانون الثاني 2021 . تم جمع العينات من المرضى من استشارية طب العيون. وقد تم تصنيف العينات إلى خمس مجموعات حسب الفئة العمرية (1-15)، (16-30)، (31-45)، (46-60)، (أكثر من 60) سنة. تضمنت الدراسة الحالية جمع العينات وتخزينها عند -20 درجة مئوية، واستخراج الحمض النووي من عينات العين باستخدام مجموعة Viral DNA Kit وفقًا لتعليمات الشركة المصنعة، وتحليل العينات باستخدام تقنيةPCR ، وإجراء التحليل الكهربائي للتحليل. يتم الحصول على نتائج تفاعل PCR وفصل الحمض النووي عن الاغاروز وذلك حسب حجم الحمض النووي. تم اكتشاف الفيروسات الغدية باستخدام طريقةPCR ، وأشارت نتائج التحليل إلى عدم وجود فيروسات غدية.
الكلمات المفتاحية: الفيروس الغدي، التهابات العين، PCR

Introduction

The eye and its accessory structure are subject to a large number of diseases that are attributed to viruses such as herpes simplex 1 and 2, adenoviruses, etc., and viruses can infect different parts of the eye.[1] Conjunctivitis is a well-known term for inflammation of the conjunctiva, which may be acute, chronic, or recurrent. Two categories of infections are used to categorize conjunctivitis: infectious conjunctivitis, which is caused by various bacterial agents like bacteria, fungi, parasites, and viruses, and non-infectious conjunctivitis caused by non-infectious causes like allergic, mechanical, chemical, immune system, toxic, and neoplastic conditions. Viral adenoviral conjunctivitis is a self-healing condition that usually lasts 2 to 3 weeks, and treatment options have been investigated to relieve symptoms or shorten infection time.[2] Although the diagnosis of viral eye disease is usually clinical, techniques used in the PCR type of confirmation such as the diagnosis of viral conjunctivitis.[3] Due to the high contagiousness of the virus, particularly adenovirus, patients with viral conjunctivitis must interrupt 5 to 2 weeks of work or school.[4] The infection may appear in crowded places, such as hospitals, schools, and mini markets[5] the most common viral infection of the outer surfaces of the eye and the conjunctiva was adenoviruses , they were also the most typical cause of viral eye infections globally, the viral type most isolated from the conjunctiva.[6] Adenoviruses are non-enveloped double-DNA viruses that were first isolated from adenovirus cells in 1953[7] they are most often the cause of acute infectious conjunctivitis.[8]

Materials and methods

A number of 180 samples from the conjunctiva, including 120 patients and 60 healthy. After PCR detection of gene amplification, a concentration of 2 agarose was utilized, the steps for the agarose gel electrophoresis method are as follows:
1 .Dissolved 2 grams of agarose in 100 ml of 1X TBE buffer in a clean beaker. 2. The solution was heated to boiling in a water bath or microwave at 100 °C for 20 minutes, when it cools down at 50-65°C, added 5µl from ethidium bromide dye .
3 .The comb was placed in a casting tray, and

agarose gel was then added and allowed to solidify for 15 minutes at room temperature.

4 .When agarose gel solidified, removed the comb carefully, the row of wells

5 .The gel was put in electrophoresis chamber, and fill out with TBE buffer until reaching 5 mm over the gel.

6. Connected the cathode to the well‟s side of the tray and the anode to another side.

Results and discussion

According to the current study, adenovirus was not found in patients with eye infections .15% to 70% of eye infections are caused on by adenoviruses.[6] Adenoviruses are the most prevalent infectious causes of conjunctivitis and represent approximately 90% of viral conjunctivitis cases.[9] The results did not agree with those of other research (Rosado-Filho)[10] 36.3% of patients had glandular conjunctivitis.[11] 100% of patients with conjunctivitis had a negative interferon test.

The findings from this study conflict with those of Balasopoulou et al.[12] who found that 134 patients had viral conjunctivitis (37.3% of men and 62.7% of women), 19 had non-specific conjunctivitis, and 3 had bacterial conjunctivitis (66.7% of men and 33.3% of women). In a recent study, it was discovered that 3.3% of patients had herpes simplex virus type 1 and 14.6% of the patients tested positive for adenovirus,[13] both studies found that patient gender was not associated with the acquisition of conjunctivitis, and increased incidence of the disease, although with advanced age, were closely associated with Mehdi et al.[14] Current study didn't agree with Li et al.[15] study for each form over three years they indicated 119 (41.0%), 125 (44.2%), and 105 (34.7%), respectively, as adenovirus the current study differed from ,some studies in other countries such as Japan during 2005-2006 (82%) study by Matsui et al. in Iran.[16]

The local study in Ramadi province - Iraq for the years 2014 and 2020 by Ahmed et al (2020) contradicted the findings of the current study, who recorded this high prevalence of patients with glandular conjunctivitis were more likely to be between the ages of 40 and adolescence than children.

According to current study's findings, adults (63%) were more likely than children (37%) to have conjunctivitis that what was confirmed by Al-Douri and Al-Jubouri.[17] Based on the present results, Ad3 and Ad4 were primarily found in patients under the age of 10; however, both were also found in individuals between the ages of 30 and 39, Adenovirus outbreaks may be occurring, as evidenced by the nearly same high incidence of adenoviruses found in the fall and summer seasons[18], while a real-time PCR analysis of 18 patients in Egypt with probable conjunctivitis revealed that 17 of the patients had enterovirus and none of the patients had adenovirus. Conjunctivitis is more common in the summer and fall when people are more likely to engage in activities like swimming and walking outside of cities, whereas in the winter when people are less likely to engage in these activities owing to the cold weather. greater potential for infection.[19]

This study's findings were in contrast to those of Schrauder *et al*.[20] whose findings indicated that acute conjunctivitis was only observed during the three years from July to October, making it impossible for them to assess the association between adenovirus and acute conjunctivitis year-round. While the frequency of conjunctivitis increased between March and June 2011 and remained the same in January, February, and July 2012.[12] According to the local study's findings by Al-Douri and Al-Jubouri,[17] the month of March saw the greatest number of cases, followed by the month of April.

Adenovirus stays rather rare throughout the year, although in September there was a recorded epidemic.[11] Another study by Maranho *et al*.[21] on the seasonal distribution of eye infections from 2004 to 2007 in Brazil found that there was just one epidemic, in April 2004, and that there was no consistency over time. Between March and May, there was a significant incidence of instances of adenoviral conjunctivitis, according to the epidemiological curve. In June, there were fewer cases overall, and in July, the outbreak came to an end. Although this epidemiological curve indicated a common source of contamination, the study's main source was not found.[22]

In contrast to the findings of Sambursky et al.[23] who discovered that from July 2003 to October 2003, adenoviral conjunctivitis was prevalent in 62% of cases, current study's findings were differen, it is believed that the seasonal variation of adenovirus, which makes it more prevalent in the summer, is responsible for the increased risk of transmission seen with summer-related activities like swimming and day camps.[24] Summer is a season when advertising is widely spotted. According to the study, fluid discharge was present in 94.1% (16 cases) of conjunctivitis patients while it was absent in all patients who were not infected with the virus, All conjunctivitis patients had 100% redness in their eyes, the best method for determining the kind of

conjunctivitis remains laboratory testing combined with clinical diagnosis.[25]

Conclusion

The current study showed that exposing *A. baumannii* isolates to temperatures equal to or less than 37c◦ enhanced biofilm formation, while exposing *A. baumannii* isolates under study to temperatures higher than 37c◦ decreased the ability of the isolates to form biofilm.The results of growing *A. baumannii* isolates on medium (T.S.B.) with a pH equal to (pH = 2, pH = 4, pH = 8.5, pH = 9, pH = 9.5, pH = 10) showed that the alkaline pH of the development medium promoted the formation of biofilm by the study isolates, where 5 isolates showed biofilm formation in an average manner at a pH equal to ph = 8, ph = 8.5, while these isolates turned into a biofilm component weakly or negatively when grown on a medium with a pH equal to pH = 2, pH = 4.

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Table 1: Prevalence of viruses associated with conjunctivitis and control group.

Cases status viral types	Patient Group		Control Group		Total	
	Positive	Negative	Positive	Negative	Positive	Negative
Adenovirus	0/0.0	120/100	0/0.0	60/100	0/0.0	180/100
$CaIX^2$= 35.685		$TabX^2$=9.49		DF=4	P. value < 0.01	

Table 2: Prevalence of adenovirus infection according to gender.

Cases status Gender	Positive Group		Negative Group		Total	
	No.	%	No.	%	No.	%
Male	0	0.0	73	60.8	73	60.8
Female	0	0.0	47	39.2	47	39.2
Total	0	0.0	120	100	120	100
$CaIX^2$=5.633		$TabX^2$=3.84		DF=1	P. value < 0.05	

Table 3: Prevalence of adenovirus in conjunctivitis group according to age groups.

Infectious status Age groups	Positive Cases		Negative Cases		Total	
	No.	%	No.	%	No.	%
1-15 years	0	0.0	34	28.3	34	28.3
16-30 years	0	0.0	25	20.9	25	20.9
31-45 years	0	0.0	34	28.4	34	28.4
46-60 years	0	0.0	19	15.8	19	15.8
Above 60 years	0	0.0	8	6.6	8	6.6
Total	0	0.0	120	100	120	100
$CaIX^2$=69.133		$TabX^2$=7.81		DF=3	P. value < 0.01	

Table 4: Prevalence of adenovirus in conjunctivitis group according to sample collection time.

Infectious status Age groups	Positive Cases		Negative Cases		Total	
	No.	%	No.	%	No.	%
October	0	0.0	15	12.5	15	12.5
November	0	0.0	46	38.3	46	38.3
December	0	0.0	38	31.7	38	31.7
January	0	0.0	21	17.5	21	17.5
Total	0	0.0	120	100	120	100
$CaIX^2$= 20.867		$TabX^2$=7.81		DF=3	P. value < 0.01	

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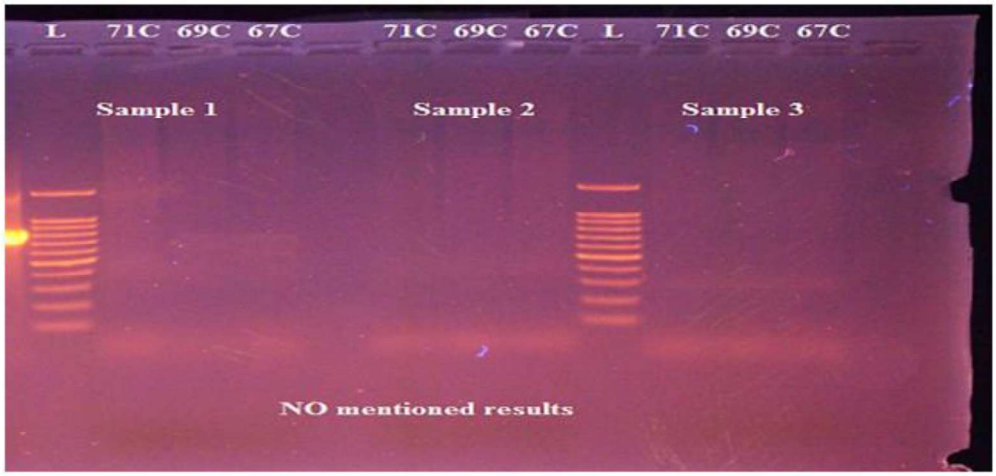

Figur1 :Gel electrophoresis for optimization process with different temperatures and different samples for PCR product of *ADENO* primer which show no results . (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with Ethidium bromide. Lane L : DNA ladder (1500-100)bp.

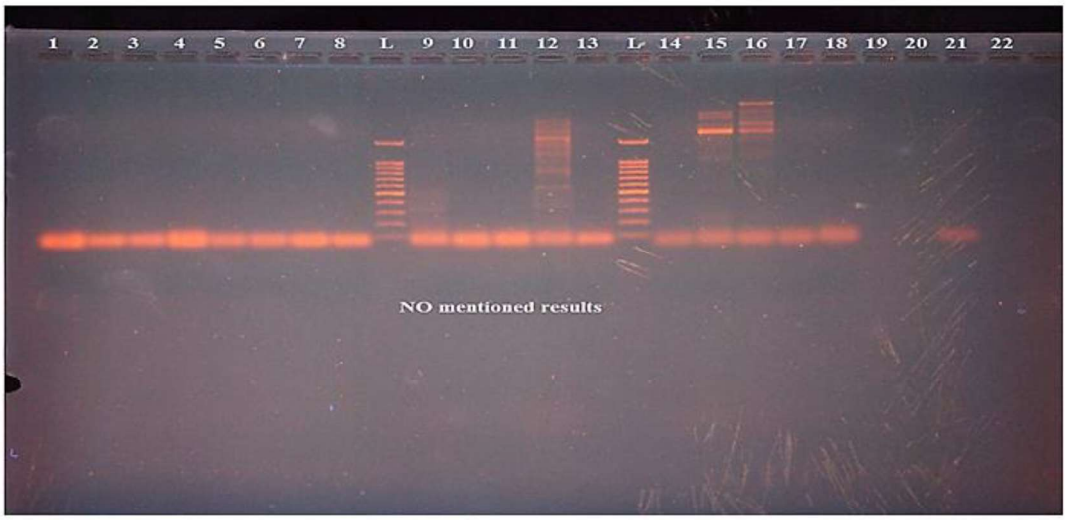

Figure 2 : Gel electrophoresis for PCR product of (*adeno* primer) show 300 bp (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with ethidium bromide. Lane L : DNA ladder (100-1500bp, all samples showed Negative results.

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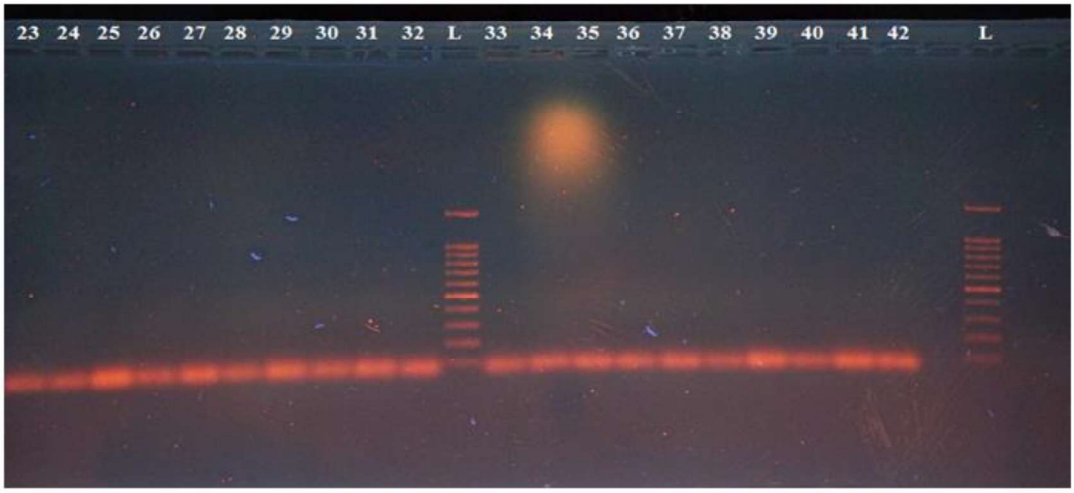

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