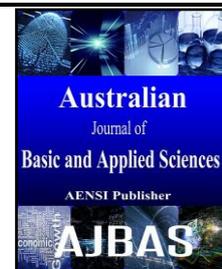




ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com



Outbreak of Epidemic Keratoconjunctivitis Caused By Human Adenoviruses in Central Iraq

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ARTICLE INFO

Article history:

Received 12 October 2014

Received in revised form 26 December 2014

Accepted 1 January 2015

Available online 27 February 2015

Keywords:

Adenovirus, Epidemic keratoconjunctivitis, Nested-PCR

ABSTRACT

Background: Epidemic keratoconjunctivitis (EKC) is caused by human adenoviruses belong to the genus Mastadenovirus of the family Adenoviridae. Human adenoviruses comprise more than 60 different serotypes/types classified into seven groups (A–G). EKC is a hyper acute and highly contagious infection of the eye caused by HAdV-D particularly, types 8, 19 and 37. More recently, HAdV-D53, D54, and D56 also have been associated with EKC. Adenovirus infections are implicated in most keratoconjunctivitis cases and it is more common in adults than children. **Objective:** The present study aims to detect human adenoviruses in conjunctival swabs of patients with keratoconjunctivitis and to clarify the significance of HAdVs in patients living in Babylon Governorate. **Results:** The results of this study showed that human adenoviruses was identified in 84 of the 93 conjunctival specimens using the nested-PCR assay, but not present in 9 specimens. EKC most frequently occurred between 21-40 years age group. EKC was predominantly in male (76.2%) from female (23.8%) and it is more common in adults (91.7%), but rarely occurred in children (8.3%). The most prevalent signs and symptoms were red eye, conjunctivitis, pain, itching tearing decreased visual acuity and photophobia were presented (100%) in all HAdVs cases, following by foreign body sensation (84.5%), chemosis (76.2%), subepithelial infiltrates (75%) bilateral (73.8%) conjunctival membrane (72.6%) pseudomembranes (71.4%) and conjunctival hemorrhage (38%). It is also noteworthy that in some cases the pharyngitis (22.6%) and rhinorrhea (21.4%) as concomitant finding. Outbreak of epidemic keratoconjunctivitis caused by HAdVs was reaching its peak in November (23.8%). **Conclusion:** The results of our study were concluded that nested-PCR technique was found to be suitable and it was simplified for application in the clinical laboratory. All age groups are susceptible for infection with HAdVs but the age groups (21-40) years old have the peak incidence of epidemic keratoconjunctivitis and the rate of outbreak EKC in male was higher than female. In addition, the highest outbreak of adenovirus has been noticed in the November month.

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To Cite This Article: Niran Kadhim F. AL-Rubaey, Azhar Amran L. AL-Tahab, Ghanim About Al-Mola, Outbreak of Epidemic Keratoconjunctivitis Caused By Human Adenoviruses in Central Iraq. *Aust. J. Basic & Appl. Sci.*, 9(7): 63-67, 2015

INTRODUCTION

EKC is the most sever type of viral keratoconjunctivitis associated with keratitis in about 80% of cases (Kanski, 2011). It is occurring mainly in adults and is typically caused by HAdV-8, -19 and -37. It has been occurring in ophthalmology clinics hospitals camps military bases and industrial plants. Human adenoviruses can remain viable for several weeks on wash basins and towels and is transmitted by contaminated ophthalmic solutions and instruments and by the sharing of bathrooms (Ruuskanen *et al.*, 2009).

EKC is one of the most common causes of acute conjunctivitis with distinctive clinical features such as sudden onset of acute follicular conjunctivitis

watery discharge hyperemia chemosis and ipsilateral pre-auricular lymphadenopathy (Gonzalez-Lopez *et al.*, 2013). EKC is typically started with a unilateral foreign body sensation and then develops within a few hours or days, into bilateral keratoconjunctivitis with marked chemosis tearing and photophobia. Visual impairment can persist for months because of sub epithelial corneal infiltrates called nummuli and irregular astigmatism (Meyer-Rüsenberg, 2011).

Diagnosis of EKC is primarily based on clinical finding. Laboratory tests to detect HAdV in conjunctival swabs including cell culture method and polymerase chain reaction (PCR) assay which are not routinely used in clinics (CDC 2013). Available rapid tests for the detection of the

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adenovirus antigen are less sensitive and less specific than nucleic acid technique, but they are useful because they can be performed easily and rapidly in clinical practice. (Siamak *et al.*, 2009).

MATERIALS AND METHODS

Patients:

Conjunctival swabs were collected from 175 randomly selected patients (age 4 to 67 years) with a mean age of 31.50 ± 15.71 years of both sexes (131 male and 44 female) suffering from keratoconjunctivitis attending the Ophthalmic Units of some hospitals in Babylon governorate were diagnosed by an experienced ophthalmologist, patients were examined under slit-lamp. The study was conducted between August 2013 and January 2014 by questionnaires papers including information about:

1. Age of patients
2. Sex of patients
3. Clinical features, including: clinical signs, clinical symptoms and concomitant finding
4. Antibiotic treatment the patients must not have any antibiotics to avoid the negative false results.

Eye swabs collection:

The conjunctival swabs were taken from the superior and inferior fornices with sterile cotton swabs without anesthesia from the affected eye of the patients, or more severe eye if both were affected. The specimens were placed in (4.5 ml) of transport media [minimum essential medium (MEM) supplemented with fetal bovine serum and antibiotics]. The specimens were frozen at -20°C until processed for PCR.

Differentiation between bacterial and viral isolates:

The conjunctival swabs were sent to the laboratory for investigation within two hours of collection. In the laboratory, they were placed and inoculated onto culture media (Blood Agar and MacConkey Agar) and incubated at 37°C for 24 hours. After that samples showed positive culture (bacterial isolates) were excluded samples showed negative bacterial culture (suspected viral isolates) were taken as study samples and stored at -20°C until use.

Extraction of DNA:

Extraction of DNA was performed by two methods the first one was done according to (Kapperud *et al.*, 1993) and modified by (Estrada *et al.*, 2007). Samples were thawed homogenized by vortexing. $50\mu\text{l}$ of sample were transferred to a 1.5 ml microcentrifuge tube and $50\mu\text{l}$ of 1X PCR buffer containing 0.2 mg of proteinase K/ml was added to each sample and mixture was vortexed and later, incubated at 37°C for 1 hour. The suspension was boiled for 10 minutes in a shaking water bath and

then centrifuged at 12500 rpm for 5 minutes at 4°C . The supernatant was used for performing the PCR. The second method was done by using DNA extraction Minikit (Qiagen) and according to manufacturer's instructions.

Detection of Adenoviruses by Nested-PCR Assay:

Viral DNA was amplified by using oligonucleotide primer pairs for detection of human adenoviruses and were selected from the DNA sequence of the open reading frame of *hex* gene. The nested-PCR protocol and primers used were as described by (Puig *et al.*, 1994) with some modifications. The primers for the first round PCR (5'-GCCGCAGTGGTCTTACATGC ACATC-3') and (5'-CAGCACGCC GCGGAT GTCAAAGT-3'). The primers for the second round of the nested-PCR (5'-GCCACCGAGACGTA CTTTCAGCCTG-3') and (5'-TTGTACGA GTACGCGGTATCCTCGCGGTC-3'). PCR products ($10\mu\text{l}$) were analysed by electrophoresis on 3% agarose gels.

Results:

Among the 175 cases of keratoconjunctivitis it was found that 82 conjunctival specimens (46.9%) showed positive culture (bacterial isolates) were excluded from the study samples, and 93 specimens (53.1%) showed negative culture (no growth) belong to other causes were taken as study samples (Table 1).

Human adenoviruses was identified in 84 of 93 conjunctival specimens by nested-PCR assay, but not present in 9 specimens and the band size of amplicon was 142bp (Table 2).

All 84 adenovirus-positive specimens from patients with epidemic keratoconjunctivitis of age groups ranging from (4-67) years were taken. All age groups were susceptible to infect with HAdVs and epidemic keratoconjunctivitis most frequently occurred between 21–40 years age group. Also, the results showed that both sexes had been affected with epidemic keratoconjunctivitis with preference of male (76.2%) from female (23.8%). A male preponderance was noted in patients of age groups ranging from 21–40 year but no difference was found in other age groups. (Table 3).

Furthermore, the results showed that epidemic keratoconjunctivitis is rarely occurring in children (8.3%) whose age groups are ($\leq 1-15$) years but it is more common in adults (91.7 %) whose age groups are (≥ 16) years (Table 4).

Clinical features were compatible with epidemic keratoconjunctivitis in patients who had visited the Ophthalmic Unit in Hospital for a related problem of eye infection. These clinical features including : clinical signs clinical symptoms, and concomitant finding. The most prevalent signs and symptoms were red eye, conjunctivitis, pain, itching tearing decreased visual acuity and photophobia were

presented in all HAdVs cases following by foreign body sensation (84.5%), chemosis (76.2%), subepithelial infiltrates (75%) bilateral (73.8%) conjunctival membrane (72.6%) pseudomembranes (71.4 %) and conjunctival hemorrhage (38%). It is also noteworthy that in some cases the pharyngitis (22.6%) and rhinorrhea (21.4%) as concomitant finding (Table 5).

Moreover, the outbreak of epidemic keratoconjunctivitis caused by HAdVs was commenced in August (6%) with increasing in the percentage to reach its peak in November (23.8%) then started to decline till it is becoming equal (20.2%) in December and January (Figure 1).

Table 1: Distribution of conjunctival swabs according to the results of bacterial culture.

Results	No. of samples	Sex		%
		Male	Female	
Positive culture	82	62	20	46.9
Negative culture	93	69	24	53.1
Total	175	131	44	100

Table 2: Nested-PCR assay to detect HAdVs in keratoconjunctivitis specimens.

Nested-PCR assay	No. of HAdVs isolation	Percentage (%)
Positive for HAdVs	84	90.3 %
Negative for HAdVs	9	9.7 %
Total	93	100

Table 3: Distribution of patients with adenoviral keratoconjunctivitis according to the age groups and sex.

Age groups (year)	No. of patients %	Sex	
		Male	Female
≤ 10	4 (4.8)	3	1
11 – 20	7 (8.3)	4	3
21 – 30	30 (35.7)	23	7
31 – 40	31 (36.9)	26	5
41 – 50	7 (8.3)	5	2
51 – 60	3 (3.6)	2	1
≥ 60	2 (2.4)	1	1
Total	84	64 (76.2%)	20 (23.8%)

Table 4: Distribution of adenoviral keratoconjunctivitis among children and adults.

Age groups (year)	Sex		Total	%
	Male	Female		
Children (≤ 1 – 15)	5	2	7	8.3
Adults ≥ 16	59	18	77	91.7
Total	64	20	84	100

Table 5: Clinical features in relation to the identified epidemic keratoconjunctivitis.

Correlation of clinical features and HAdVs		
Clinical signs	No. of cases	%
Red eye	84	100
Conjunctivitis	84	100
Chemosis	64	76.2
Conjunctival hemorrhage	32	38
Pseudomembranes	60	71.4
Conjunctival membrane	61	72.6
Subepithelial infiltrates	63	75
Clinical symptoms		
pain	84	100
Itching	84	100
Tearing	84	100
Foreign body sensation	71	84.5
Decreased visual acuity	65	77.4
Photophobia	84	100
bilateral	62	73.8
Concomitant finding		
Rhinorrhea	18	21.4
Pharyngitis	19	22.6

Dicussions:

Viral keratoconjunctivitis is a common highly contagious disease that mainly involves the surface of the eye. It is caused by adenoviruses that are highly resistant to environmental influences (Adloch *et al.*, 2010).The use of nested-PCR was crucial to

improve the sensitivity of the assay, the two-step reaction can detect very low numbers of adenovirus particles and has the advantage of decreasing the concentrations of PCR inhibitors (Rigotto *et al.*, 2005).

The results of the present study showed that epidemic keratoconjunctivitis more occurred in male (76.2%) than female (23.8%). Tasman and Jaeger, (2001) and in South of Saudi Arabia, Abdelkader, (2014) were stated that adenovirus is responsible for keratoconjunctivitis in patients with both sexes without any preference regarding sex.

Also, The results showed that epidemic keratoconjunctivitis more common in adults (91.7%) and is rarely occurring in children (8.3%), it is maybe the outbreak chanced to be in factories, government departments and institutions with large populations in which their working places were closed and crowded. On the other hand, bad hygienic habits and environments, increased transmission quickly. These results were corresponded to those outcomes published by results of studies Tasman and Jaeger, (2001) ; Adloch *et al.*, (2010) were reported that epidemic keratoconjunctivitis can be affected all age groups, and the infection is more common in adults.

Epidemic keratoconjunctivitis outbreaks have been reported in this study with almost similar sign and symptoms were reported in another study Reuter *et al.*, (2007) who mentioned that, the main clinical symptoms in patients with keratoconjunctivitis including conjunctivitis (100%), lacrimation (94%), foreign body sensation (83%), and decreased visual acuity (76%). In the current study, the appearance of subepithelial infiltrates is additional clinical sign which can be observed in 75% and the rate of bilateral cases was 73.8% of epidemic keratoconjunctivitis cases. This result corresponds to the results of Abdelkader, (2014) who stated that subepithelial infiltrates involving the visual axis were spotted in 76% of cases causing photophobia and deterioration of visual acuity in these patients and epidemic keratoconjunctivitis was observed in both eyes in up to 70% of the cases.

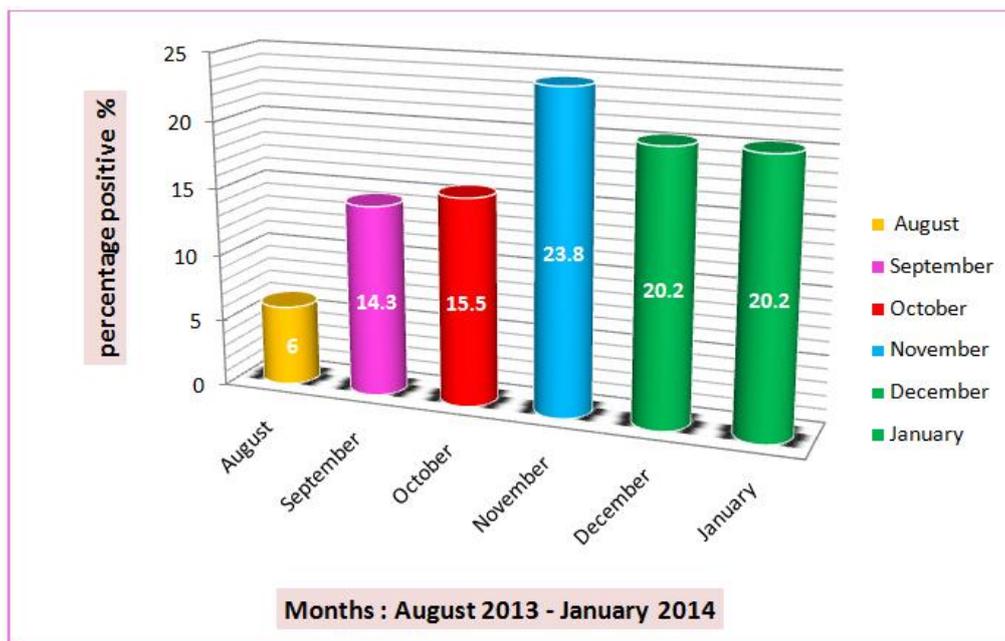


Fig. 1: Distribution of HAdVs positive patients for the period from August 2013 to January 2014

In addition, the results of present study showed the conjunctival membrane and pseudomembranes were up to (72.6%) and (71.4%), respectively. Pseudomembranes are clots of exudates firmly adhered to the upper and lower tarsal conjunctivae. It is possible to separate them from the underlying conjunctiva without damaging the epithelium by means of peeling, producing minor bleeding, if any (González-López *et al.*, 2013).

Furthermore, outbreak keratoconjunctivitis caused by HAdVs was commenced in August with the peak reaching in November with a slow decline in the percentage to become equal in December and January. This high incidence might be due to collection of most samples during the cold months.

This result disagreed with the results recorded in Pakistan by Majeed *et al.*, (2005) who observed that the highest incidence of adenoviral conjunctivitis in the month of July. In *Chennai, India*, Janani *et al.*, (2012) stated that HAdVs responsible for keratoconjunctivitis was commenced in August with the peak reaching in September with a slow decline in the number in October.

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