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Original Article

The relationship between clinical findings and viral load in Adenoviral Keratoconjunctivitis

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Abstract

The study aimed to evaluate the relationship between clinical findings and viral load in adenoviral keratoconjunctivitis (Ad-Kc). In this cross-sectional study, 30 eyes of 30 patients with Ad-Kc were enrolled. The real-time polymerase chain reaction was performed to detect and quantify adenovirus in all samples. The patient groups were divided into three subgroups according to baseline viral loads ($<10^7$, 10^7-10^8, $>10^8$ HAdV copies/ml). The follow-up duration, HAdV DNA copy number, treatment regimen, and detailed clinical findings including uncorrected visual acuity, eyelid edema, conjunctival hyperemia, chemosis, follicular reaction, corneal involvement, conjunctival pseudomembrane, and subepithelial infiltrates (SEIs) were recorded. The study showed that a high initial viral load was associated with the development of SEIs and pseudomembrane formation ($p<0.05$). Clinical findings and ocular complications of Ad-Kc were found similar in treatment groups at the final visit ($p>0.05$). Our results showed that a high initial viral load in Ad-Kc may be predictive of inflammatory sequelae. Determination of the initial viral load in Ad-Kc may be helpful to better understand the clinical course of the disease and prevent complications.

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Introduction

Viruses cause about 80% of all cases of acute conjunctivitis and human adenoviruses (HAdVs) are believed to account for approximately 65–90% of the viral conjunctivitis cases (1,2). Adenoviral keratoconjunctivitis (Ad-Kc) is a public health concern due to its highly contagious nature and is associated with epidemic outbreaks (3,4).

It is a follicular conjunctivitis which is thought of as a self-limiting disease with no long-term sequelae, however in severe cases, life-changing chronic inflammatory complications and decrease in vision can happen (5). While pseudomembrane formation can cause corneal erosions, symblepharon, and dry eye; subepithelial deposits (SEIs) can reduce visual acuity (VA) from a few months to several years (6-7).

Currently, there is no approved treatment for Ad-Kc, whose therapeutic options are limited to supportive therapies and palliative measures (4,8). A therapeutic agent that reduces the clinical symptoms and signs of Ad-Kc and minimizing the spreading would be desirable. Treatment should generally target the symptoms and ancillary effects of the virus. Povidone Iodine (PVP-I), artificial tears, and sometimes anti-inflammatory agents can be used as treatment options (9-14). Therefore, the main goals of clinical management should be to identify severe cases, prevent or limit the spread, provide symptomatic relief, and follow-up closely for developing complications (1,15,16).

In previous studies, it has been reported that there is a correlation between HAdV load in respiratory tract secretions and disease severity in patients with HAdV pneumonia.

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Therefore, it has been suggested that the HAdV load in respiratory tract infections may help clinicians identify severe cases (17,18).

In this study, it was aimed to investigate the relationship between initial viral load and clinical findings and inflammatory complications in Ad-Kc. As a secondary aim, the effect of topical use of 0.15% GCV (Virgan ®; Farmila-Thea, Milan, Italy) on the clinical course in Ad-Kc was sought.

Material and Methods

This study was a randomized controlled clinical study involving patients with Ad-Kc conducted at Cukurova University Department of Ophthalmology. The study was adhered to the tenets of the Declaration of Helsinki.

The initial diagnosis of Ad-Kc was made by clinical examination and ocular swab specimens were taken to confirm the diagnosis. Exclusion criteria were as follows: patients with any other ocular anterior segment morbidity, using any topical ocular medication, pregnancy or breastfeeding, comorbidities that may affect the immune system function, and those who did not attend the follow-up examination.

Conjunctival swabs were taken from the inferior palpebral conjunctiva after the instillation of a topical anesthetic. All specimens were sent to the Medical Virology Department for HAdV by real-time polymerase chain reaction (PCR) analysis. The presence of HAdV DNA in conjunctival specimens was quantitatively investigated using Bosphore Adenovirus Quantification Kit v1 (Anatolia gene works, Turkey). The kit performs the quantitative detection of adenovirus by amplifying a part of the hexon gene. Adenovirus real-time

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PCR was carried out at 97 C for 14:30 min of initial denaturation, followed by 50 cycles of 97 C for 30 sec, 53 C for 1:30 min and 72 C for 15 sec in a Montania 4896 real-time PCR instrument. The increase in the FAM channel is considered positive for HAdV, while the increase in the HEX channel indicates the positivity of the internal control and the extraction was done properly.

As a routine practice in our clinic, instilled one drop of proparacaine followed by four-five drops of 5% PVP-I was performed only once to all patients after were taken a conjunctival swab for PCR detection of HAdV.

The follow-up duration, real-time PCR test results, HAdV DNA copy number, treatment regimen, detailed clinical findings [including uncorrected VA (UCVA), eyelid edema, conjunctival hyperemia, chemosis, follicular reaction, corneal involvement, conjunctival pseudomembrane, and SEIs were recorded. The patient groups were divided into three groups according to baseline viral loads ($<10^7$, 10^7-10^8, $>10^8$ HAdV copies/ml). In addition, patients were randomly divided into 2 groups according to the treatment given. Fifteen patients (50%) were given topical AT (four times a day) (Group 1) and 15 patients (50%) were given topical 0.15% GCV ophthalmic gel (four times a day) + AT (Group 2). Data collected at baseline and final visits were analyzed for groups.

Statistical analysis

Wilcoxon signed-rank test was used the compare baseline variables between the groups. Generalized linear mixed model and chi-square test were used to evaluate the homogeneity of categorical variables before and after treatment between the two groups. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package**.** A level of $P<0.05$ was accepted as statistically significant.

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Results

A total of 30 eyes of 30 patients (16 men, 14 women) who were diagnosed with Ad-Kc for less than 3 days from the onset were enrolled in the study. The mean patient age was 31.8 ± 21.9 years (range 18-69 years). The mean follow-up duration was 12.8 ± 2.1 days (10-15 days). At baseline, all cases had unilateral involvement except 3 cases. When patients were evaluated in groups according to initial viral load, the development of SEIs and pseudomembrane formation were significantly higher in eyes with a viral load above 10^7 copies/ml ($p<0.001$, for both). However, there was no significant relationship between other clinical findings and initial viral load at the final visit ($p>0.05$ for all, Table 1).

Compared with baseline, the visual loss was observed in 11 patients at the final visit (mean Snellen line loss 2 ± 0.6, 1.8 ± 0.8, p=0.32 respectively). All of these eyes had SEI at the final visit.

There was no statistically significant difference between the two treatment groups in their baseline characteristics and viral load (Table 2). The presence of conjunctival hyperemia, lid edema, chemosis, follicular reaction, and corneal involvement was similar in both groups at baseline ($p>0.05$, for all findings) (Table 3).

There was no statistically significant difference between the groups in the effect of the given treatment on the improvement in the clinical findings at the final visit ($p>0.05$ for all findings) (Table 3). As shown in Table 4, there were also no statistical differences between the groups for subepithelial infiltrates (SEI), conjunctival pseudomembrane, and fellow eye involvement at the final visit (p=0.34, p=0.94, p= 0.70, respectively). The mean UCVA of both

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groups was similar at the final examination (Group 1; 0.77 ± 0.16, Group 2; 0.84 ± 0.18, p=0.28).

Discussion

In addition to the uncomfortable acute clinical symptoms, Ad-Kc can be further complicated by chronic inflammatory complications that limit patients' daily activities (19).

In some cases with Ad-Kc, mucopurulent conjunctival discharge forms a mass in the tarsal conjunctiva of fibrin and collagen engulfing macrophages, neutrophils, and lymphocytes. These fibrin-rich layers of exudate adhering to the tarsal conjunctiva are pseudomembrane formations (20). Pseudomembranous conjunctivitis can lead to epithelial corneal erosions that ocur due to friction from the rough membrane during blinking. Additionally, tarsal inflammation and bleeding may cause subconjunctival scarring, lacrimal drainage abnormalities, dry eye, and symblepharon (21).

Subepithelial infiltrates are believed to represent a cellular immune reaction against viral antigens, deposited in the corneal stroma (22). Histopathologic evaluation of SEIs has shown that they are composed of lymphocytes, histiocytes, and fibroblasts accompanied by a disruption of the collagen la, and are thought to be the result of delayed immune response to viral antigens in the corneal stroma (23). Persistent corneal SEIs, one of the late inflammatory complications of the disease, especially developing in 40-50% of patients, may cause vision loss, glare, and photophobia (24). Given the same pathophysiological origin of pseudomembranes and SEIs, it has been suggested that pseudomembranous conjunctivitis may also be associated with SEIs (6). Consistent with the previous study, the rate of cases with SEIs and pseudomembrane was found similar in this study (53% vs. 40%).

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In the current study, high initial viral load was associated with the development of SEIs and pseudomembrane formation in patients with Ad-Kc. This may suggest earlier use of topical anti-inflammatory agents such as corticosteroids and Cyclosporin A in patients with an initial high viral load in clinical practice.

Lee et al. showed that in cases with Ad-Kc, high initial viral load was only predictive of the development of SEIs but was not associated with age, gender, or treatment is given.

In the subanalysis of the HAdV species, they also showed that people infected with HAdV-D were significantly more likely to have a persistent viral infection and develop SEIs compared to patients infected with other HAdV species (25).

Since there is no specific drug against HAdV, the usual treatment of Ad-Kc is primarily supportive and includes AT and cold compresses (26,27). Although topical GCV decreases HAdV load experimentally, there are few studies about its efficacy in the treatment of Ad-Kc in clinical trials (28,29). As a secondary outcome of this study, we sought the effect of GCV on clinical findings, as some patients received topical GCV treatment. In the current study, no statistically significant difference was found between the treatment groups in the clinical findings and ocular complications of Ad-Kc.

Adenoviral keratoconjunctivitis is a biphasic disease, beginning with the infectious phase, which is followed by the inflammatory phase. The onset of HAdV conjunctivitis may seem rapid, but in reality, there is an incubation period of about one week before the clinical symptoms present (4). The acute infectious phase of the disease is marked by severe conjunctivitis and lasts from two to four weeks. After conjunctivitis appears, there is a period of viral shedding where the self-limiting virus is gradually cleared from its host. The RAPID trial and placebo-controlled studies provided evidence that inflammatory sequelae may persist despite clearance of viral titers after 7 days of follow-up in the natural course of

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untreated eyes with Ad-Kc (10,11,25). Considering that many patients wait for symptom onset to seek care, this may reduce the effectiveness of any treatment that has an inhibitory effect on HAdV. Thus, a relatively late suppression of virus replication by GCV may have a limited effect on the recovery of clinical findings during the inflammation period after the signs of acute conjunctivitis appear. Moreover, Maatsura and colleagues showed that the viral load in healed eyes on day 15 was significantly higher than in unhealed eyes. They suggested that this was likely due to the administration of corticosteroids to their all-study eyes, which relieved symptoms and suppressed inflammation, but prolonged the viral shedding period (30). In the light of all information mentioned above, it can be concluded that the decrease in inflammation rather than the decrease in viral load may be effective in the prevention of inflammatory complications of Ad-Kc.

Limitations of the study were the small sample size and lack of type identification for HAdV. Therefore we did not evaluate the possible association of virus type with clinical findings in the study.

In conclusion, our results indicated that high initial viral load in Ad-Kc may be predictive of inflammatory sequelae such as pseudomembrane formation and SEIs. By determining the initial high virus load, severe cases can be predicted and complications can be minimized with close follow-up and early anti-inflammatory treatment.

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Conflict of Interest

The authors declare that they have no conflict of interest

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Table 1. Comparison of the final clinical findings according to viral load subgroups

Clinical findings (%,n)	**real-time PCR (copies/ml)**			
	$<10^7$	**10^{7-} 10^8**	**$>10^8$**	**P value**
Conjunctival hyperemia	50%(5)	40%(4)	40%(4)	0.87
Eyelid edema	33.3%(3)	60%(6)	33.3%(3)	0.29
Chemosis	33.3%(3)	40%(4)	40%(4)	0.86
Follicular reaction	60%(6)	60%(6)	70%(7)	0.87
Punctate corneal epitheliopathy	20%(2)	20%(2)	40%(4)	0.51
Conjunctival pseudomembrane	-	60%(2)	100% (10)	**<0.001**
Subepithelial infiltrate	-	60%(6)	100%(10)	**<0.001**

PCR; polymerase chain reaction

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Table 2. Demographics and baseline characteristics

	Group 1 (n=15)	**Group 2 (n=15)**	**P value**
Age	35.7 ± 21.1	29.3 ± 19.3	0.25
Gender	7 /8 (M/F)	9/6 (M/F)	0.08
UCVA	0.850.2	0.91 ± 0.1	0.6
Real-time PCR (copies/ml) • **$<10^7$** • **10^7-10^8** • **$>10^8$**	2561 x 10^6 (1621-22x10^9) n=4 n=5 n=6	1826 x 10^6 (1700-2x10^9) n=6 n=5 n=4	0.67
Follow-up time (days)	13.1 ± 2.1	12.5 ± 2.5	0.16

UCVA; uncorrected visual acuity, PCR; polymerase chain reaction

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Table 3. Comparison of clinical findings in both treatment groups at baseline and at the final visit

	At baseline			At final visit		
Clinical findings % (n)	**Group 1 (n=15)**	**Group 2 (n=15)**	**P value**	**Group 1 (n=15)**	**Group 2 (n=15)**	**P value**
Conjunctival hyperemia	100% (15)	100% (15)	0.4	40% (6)	46.6% (7)	0.65
Eyelid edema	73.3% (11)	93.3% (14)	0.88	40% (6)	40% (6)	0.45
Chemosis	46.6% (7)	66.6% (10)	0.3	33.3% (5)	40% (6)	0.68
Folicular reaction	66.6% (10)	66.6% (10)	0.68	53.3% (8)	73.3% (11)	0.16
Punctate corneal epitheliopathy	40% (6)	26.6% (4)	0.53	33.3% (5)	20% (3)	0.9

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Table 4. Comparison of ocular complications in both groups

	Group 1 (n=15)	**Group 2 (n=15)**	**P value**
Conjunctival pseudomembrane	33.3% (5)	46.6% (7)	0.45
Subepithelial infiltrate	60% (9)	46.6% (7)	0.53
Fellow eye involvement	50% (7)*	38.4% (5)*	0.70

*n=14 for group 1; n=13 for group 2

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