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Long-term effects of adenoviral keratoconjunctivitis on conjunctival goblet cell density and tear film parameters: a prospective case-control study

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Abstract

Background Adenoviral keratoconjunctivitis (AKC) is a common ocular infection that causes acute inflammation of the ocular surface. However, the long-term effects of AKC on conjunctival goblet cell density and tear film stability remain poorly understood. This study aimed to evaluate the long-term impact of AKC on goblet cell morphology, density, and tear film parameters.

Methods This prospective case-control study included 95 eyes of 64 patients with a history of AKC diagnosed during epidemic outbreaks based on strict clinical criteria (mean follow-up: 18.3 ± 6.8 months after the acute episode) and 92 eyes of 55 age-matched healthy controls. All participants underwent Schirmer test with anesthesia, tear breakup time (TBUT) measurement, slit-lamp evaluation of lid margin and meibomian gland function, and conjunctival impression cytology. Goblet cell density was quantified, and epithelial changes were graded using the Nelson classification system. Adenovirus serotyping was performed by multiplex PCR in a subset of patients during the acute phase. Post-hoc power analysis and multivariable logistic regression adjusting for sex and age were performed.

Results The mean age was 45.2 ± 14.6 years in the case group and 42.8 ± 13.9 years in the control group ($p = 0.312$). Goblet cell density was significantly lower in the AKC group ($116 [58-289]$ cells/mm²) compared with controls ($463 [123-1063]$ cells/mm²; $p < 0.001$), representing a 75% reduction. The median Nelson score was higher in the AKC group ($2.0 [1.0-2.0]$) than in controls ($1.0 [0.0-1.75]$; $p < 0.001$), indicating more advanced squamous metaplasia. Schirmer test values ($10.0 [8.0-13.0]$ vs. $14.0 [10.25-17.0]$ mm; $p < 0.001$) and TBUT (8.16 ± 2.98 vs. 11.1 ± 3.20 s; $p < 0.001$) were significantly reduced in the AKC group. Adenovirus serotype 8 was identified in 80% of typed samples. Sex-stratified analysis confirmed significant goblet cell reduction in both sexes. Multivariable regression confirmed AKC as an independent predictor of goblet cell loss after adjusting for sex and age (adjusted OR: 4.82; 95% CI: 2.61–8.91; $p < 0.001$).

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Conclusions AKC leads to persistent goblet cell loss and squamous metaplasia, resulting in decreased tear film stability and dry eye symptoms at 18 months post-infection, independent of sex and age. Future studies should incorporate validated patient-reported outcome measures to fully characterise the symptomatic burden of post-AKC ocular surface disease.

Keywords Adenoviral keratoconjunctivitis, Dry eye disease, Goblet cells, Impression cytology, Tear film, Squamous metaplasia, Epidemic keratoconjunctivitis

Introduction

Adenoviruses are the most common cause of viral conjunctivitis worldwide, accounting for 65–90% of viral ocular infections [1, 2]. Clinical manifestations include epidemic keratoconjunctivitis (EKC), pharyngoconjunctival fever (PCF), and acute nonspecific follicular conjunctivitis [3]. Among these, EKC is the most severe form, characterized by intense inflammation, pseudo-membrane formation, and subepithelial corneal infiltrates that may persist for months to years [4].

Following adenoviral keratoconjunctivitis, the ocular surface undergoes a spectrum of changes that may persist well beyond clinical resolution of the acute infection. Cytopathic damage to the conjunctival epithelium during the acute phase, combined with the intense inflammatory response characteristic of epidemic keratoconjunctivitis, has been implicated in the depletion of conjunctival goblet cells and the induction of squamous metaplasia [5, 6]. Post-infectious dry eye syndrome has been reported in approximately one third of patients following epidemic keratoconjunctivitis [7], yet the long-term quantitative impact on goblet cell density, tear film parameters, and conjunctival epithelial morphology has not been systematically characterised in a prospective case-control design.

Conjunctival goblet cells play a crucial role in maintaining ocular surface homeostasis by secreting gel-forming mucins, particularly MUC5AC, which provides a protective mucous layer over the corneal and conjunctival epithelium [8]. Reduced goblet cell density has been associated with tear film instability and is considered a hallmark of ocular surface disease [9, 10]. Impression cytology is a minimally invasive technique that allows for the evaluation of conjunctival epithelial cell morphology, goblet cell density, and the degree of squamous metaplasia [11].

Although the acute effects of adenoviral infection on the ocular surface are well documented, the long-term consequences on the conjunctival goblet cell population and tear film function remain poorly understood. Previous studies have focused primarily on corneal complications such as subepithelial infiltrates, while the impact on conjunctival goblet cells has received limited attention [12, 13]. We hypothesized that AKC causes persistent goblet cell loss and squamous metaplasia, contributing to chronic dry eye symptoms. The aim of this study was

to evaluate goblet cell density, morphology, and tear film parameters in patients with a history of AKC compared with healthy age-matched controls at a mean follow-up of 18 months after the acute infection.

Materials and methods

Study design and participants

This prospective case-control study was conducted at the Ophthalmology Department of Kocaeli University Faculty of Medicine. The study protocol was approved by the institutional ethics committee (Decision No: 2016/13.9) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

The case group consisted of 95 eyes from 64 patients who had been diagnosed with AKC at our institution and were evaluated at a mean of 18.3 ± 6.8 months (range: 12–32 months) after the acute episode. The diagnosis of AKC during the acute phase was based on the presence of at least three of the following characteristic clinical features: (1) acute follicular conjunctival reaction (2), conjunctival hyperemia with watery discharge (3), ipsilateral preauricular lymphadenopathy (4), lid edema and/or chemosis, and (5) subepithelial corneal infiltrates (SEIs) appearing within 1–3 weeks of onset. The requirement for at least three of five predefined criteria was established a priori based on published clinical diagnostic frameworks for epidemic keratoconjunctivitis [14]. In epidemic settings, the combination of follicular conjunctival reaction, preauricular lymphadenopathy, and subepithelial corneal infiltrates carries a high positive predictive value for adenoviral aetiology. All cases in the current cohort were identified during documented institutional outbreaks, which substantially increases the prior probability of adenoviral causation and the clinical accuracy of diagnosis [15].

All cases were diagnosed during documented epidemic outbreaks at our institution between 2016 and 2017, where clusters of patients presented with the classic EKC phenotype, which substantially increases the positive predictive value of clinical diagnosis for adenoviral etiology [1].

To minimize the risk of diagnostic misclassification, patients with features suggestive of alternative etiologies were excluded: mucopurulent or purulent discharge (suggestive of bacterial conjunctivitis), predominantly

papillary conjunctival reaction with itching and seasonal pattern (suggestive of allergic conjunctivitis), unilateral presentation without follicular response (suggestive of other inflammatory etiologies), and concurrent systemic symptoms without the full EKC phenotype. It should be noted that clinical diagnosis remains the standard approach for AKC in routine practice, as rapid antigen detection tests have sensitivities ranging from 39% to 89% depending on the clinical setting and PCR-based confirmation is not widely available during epidemic outbreaks [16, 17].

In a subset of patients ($n=6$), the diagnosis was confirmed by polymerase chain reaction (PCR) detection of adenoviral DNA from conjunctival swabs during the acute phase. The control group consisted of 92 eyes from 55 healthy individuals with no history of AKC or other ocular surface diseases. Control subjects were recruited from patients attending the clinic for routine refractive evaluation.

Inclusion and exclusion criteria

Inclusion criteria for the case group were: (1) documented history of AKC diagnosed at our institution during the 2016–2017 epidemic period (2), minimum follow-up of 12 months after the acute episode, and (3) age ≥ 18 years. Inclusion criteria for the control group were: (1) no history of viral conjunctivitis (2), no symptoms or signs of dry eye disease, and (3) age ≥ 18 years.

Exclusion criteria for both groups were: (1) history of other ocular surface diseases (e.g., allergic conjunctivitis, Stevens-Johnson syndrome, ocular cicatricial pemphigoid) (2), previous ocular surgery (3), contact lens wear within the past 3 months (4), use of topical ophthalmic medications other than preservative-free artificial tears within the past 4 weeks, specifically including topical corticosteroids, immunosuppressive agents (cyclosporine A, tacrolimus), and non-steroidal anti-inflammatory drugs (5), systemic diseases known to affect the ocular surface (e.g., Sjögren syndrome, rheumatoid arthritis, diabetes mellitus) (6), clinical evidence of meibomian gland dysfunction (defined as gland dropout, orifice plugging, or abnormal meibum quality on slit-lamp evaluation) (7), lid abnormalities, and (8) pregnancy or lactation.

The use of preservative-free artificial tears (Refresh Tears PF, 0.5% carboxymethylcellulose sodium; Allergan, Dublin, Ireland) was permitted in both groups, as withholding lubrication in symptomatic post-AKC patients would not be ethically justifiable. The type, frequency, and duration of artificial tear use were documented for all participants.

Ophthalmic examination

All participants underwent a comprehensive ophthalmic examination including best-corrected visual

acuity (BCVA), slit-lamp biomicroscopy, and fundus examination. Slit-lamp evaluation of the lid margin was performed in all participants, including assessment of meibomian gland orifice morphology and manual expression of the central lower lid meibomian glands to evaluate the quality and ease of meibum expression. Tear film assessment was performed in the following order to minimize test interference: tear breakup time (TBUT), followed by Schirmer test with anesthesia.

Tear breakup time (TBUT)

TBUT was measured by instilling fluorescein sodium dye into the inferior fornix using a sterile fluorescein strip moistened with preservative-free saline. Patients were instructed to blink naturally three times and then keep their eyes open. The tear film was observed under slit-lamp examination using cobalt blue illumination. The time interval between the last complete blink and the appearance of the first dry spot on the cornea was recorded. Three consecutive measurements were obtained, and the average value was used for analysis. A TBUT of less than 10 s was considered abnormal [18].

Schirmer test

Schirmer test with topical anesthesia (Schirmer II test) was performed to assess basal tear secretion. One drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Fort Worth, TX, USA) was instilled into the inferior fornix of each eye. After 1 min, excess fluid was gently blotted from the inferior fornix without touching the cornea. Standardized Schirmer strips (5 mm $\times$ 35 mm; Whatman No. 41 filter paper) were placed at the junction of the middle and lateral thirds of the lower eyelid. Patients were instructed to keep their eyes gently closed during the test. After 5 min, the strips were removed, and the length of wetting was measured in millimeters from the notch. A Schirmer value of less than 10 mm was considered abnormal [19].

Conjunctival impression cytology

Impression cytology samples were obtained from the temporal bulbar conjunctiva after topical anesthesia with 0.5% proparacaine hydrochloride. Cellulose acetate filter paper (Millipore HAWP304; pore size 0.45 μ m) was cut into 3 $\times$ 4 mm strips. Using smooth forceps, the filter paper was applied to the temporal interpalpebral conjunctiva, approximately 2–3 mm from the limbus, with gentle pressure for 3–5 s. The paper was then carefully peeled off and immediately placed in fixative solution (glacial acetic acid, 37% formaldehyde, and 70% ethyl alcohol in a ratio of 1:1:20).

Specimens were stained with periodic acid-Schiff (PAS) and hematoxylin according to standard protocols. Slides were examined by an experienced pathologist who was

masked to the clinical diagnosis. Goblet cells were identified by their characteristic PAS-positive cytoplasm and counted in four randomly selected high-power fields ($\times 400$ magnification). The goblet cell density was calculated as the number of cells per mm^2 (one high-power field at $\times 400$ magnification = 0.24 mm^2).

The degree of conjunctival squamous metaplasia was graded using the Nelson classification system [20]: Grade 0: small, round epithelial cells with large nuclei; abundant plump, oval goblet cells. Grade 1: slightly larger, polygonal epithelial cells with smaller nuclei; slightly reduced goblet cells that remain oval. Grade 2: larger, polygonal epithelial cells, occasionally multinucleated, with small nuclei; markedly reduced, smaller goblet cells. Grade 3: large, polygonal epithelial cells with pyknotic nuclei; complete absence of goblet cells.

Adenovirus detection and serotyping

In a subset of six patients who presented during the acute phase of AKC, conjunctival swab samples were collected for virological analysis. Swabs were obtained using nylon-flocked swabs (UTM Kit; Copan Diagnostics, Murrieta, CA, USA) and transported to the laboratory on ice within 2 h. DNA was extracted using a commercial kit (Ribospin vRD; GeneAll, Seoul, Korea) according to the manufacturer's instructions.

Adenovirus DNA was detected using PCR with primers targeting a conserved region of the hexon gene, as previously described [21]. Serotyping of adenovirus-positive samples was performed using multiplex PCR with serotype-specific primers for types 3, 4, 7, 8, 19, and 37, which are the most common ocular adenovirus serotypes [22].

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous variables are presented as mean $\pm$ standard deviation (SD) and were compared using the independent samples t-test. Non-normally distributed variables are presented as median (interquartile range [IQR]) and were compared using the Mann-Whitney U test. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. A two-tailed p-value < 0.05 was considered statistically significant. Since some participants contributed both eyes to the analysis, generalized estimating equations (GEE) with an exchangeable correlation structure were used to account for the intra-individual correlation between fellow eyes. The patient identifier was specified as the clustering variable. Given that all primary comparisons yielded p-values < 0.001 , the results remained significant even after Bonferroni correction for multiple comparisons (adjusted significance threshold: $p < 0.0125$ for four primary outcomes).

Post-hoc power analysis was performed using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Germany) [23]. For the primary outcome (goblet cell density), the nonparametric effect size was estimated from the Mann-Whitney U statistic using the formula $r = Z/\sqrt{N}$, and subsequently converted to Cohen's d equivalent using $d = 2r/\sqrt{1 - r^2}$. Power was calculated at an alpha level of 0.05. For TBUT, which was normally distributed, Cohen's d was calculated directly from the observed means and pooled standard deviation.

To evaluate the potential confounding effect of sex on the primary outcomes, sex-stratified subgroup analyses were performed. Additionally, binary logistic regression analysis was conducted with dichotomized goblet cell density (above vs. below median) as the dependent variable and group assignment (AKC vs. control), sex, and age as independent variables to assess the independent contribution of AKC history after adjusting for potential confounders. A subgroup analysis within the AKC group was also performed to evaluate the potential confounding effect of artificial tear use on tear film and impression cytology outcomes.

Results

Demographic and clinical characteristics

A total of 64 patients (95 eyes) in the case group and 55 individuals (92 eyes) in the control group were included in the study. The demographic and clinical characteristics of the study population are summarized in Table 1. There was no statistically significant difference in age between the two groups (45.2 ± 14.6 vs. 42.8 ± 13.9 years; $p = 0.312$). The proportion of females was higher in the case group

Table 1 Demographic and clinical characteristics of the study population

Parameter	AKC Group (n = 64)	Control Group (n = 55)	p-value
Age (years), mean $\pm$ SD	45.2 $\pm$ 14.6	42.8 $\pm$ 13.9	0.312
Female, n (%)	41 (64.1%)	23 (41.8%)	0.014*
Eyes included, n	95	92	—
Follow-up (months), mean $\pm$ SD	18.3 $\pm$ 6.8	N/A	—
Schirmer test (mm), median (IQR)	10.0 (8.0–13.0)	14.0 (10.25–17.0)	$< 0.001^*$
TBUT (seconds), mean $\pm$ SD	8.16 $\pm$ 2.98	11.1 $\pm$ 3.20	$< 0.001^*$
Nelson score, median (IQR)	2.0 (1.0–2.0)	1.0 (0.0–1.75)	$< 0.001^*$
Goblet cell density (cells/ mm^2), median (IQR)	116 (58–289)	463 (123–1063)	$< 0.001^*$

AKC, adenoviral keratoconjunctivitis; IQR, interquartile range; SD, standard deviation; TBUT, tear breakup time. *Statistically significant ($p < 0.05$)

(64.1%) than in the control group (41.8%; $p=0.014$). The mean follow-up duration after the acute AKC episode was 18.3 ± 6.8 months (range: 12–32 months). In the AKC group, 42 of 64 patients (65.6%) reported regular use of preservative-free artificial tears at the time of assessment, while no control subjects reported artificial tear use.

Statistical power

Post-hoc power analysis revealed that the study achieved a statistical power exceeding 99% for the primary outcome (goblet cell density), based on an observed non-parametric effect size of $r=0.39$, corresponding to a Cohen's d equivalent of 0.85 (large effect), with 95 eyes in the case group and 92 eyes in the control group at an alpha level of 0.05. For TBUT (normally distributed), Cohen's d was calculated directly as 0.95 (mean difference: 2.94 s; pooled SD: 3.09 s), yielding a power of >99%. For Schirmer test values, the estimated effect size was $d=0.91$, with power >99%. These results confirm that the study sample was adequately powered to detect the observed differences across all primary outcome measures.

Tear film parameters

The median Schirmer test value was significantly lower in the AKC group (10.0 mm [IQR: 8.0–13.0]) compared with the control group (14.0 mm [IQR: 10.25–17.0]; $p<0.001$). Similarly, the mean TBUT was significantly reduced in the AKC group (8.16 ± 2.98 s) compared with controls (11.1 ± 3.20 s; $p<0.001$). In the AKC group, 52.6% of eyes (50/95) had Schirmer values below 10 mm, and 67.4% (64/95) had TBUT values below 10 s (Fig. 1a, b).

Impression cytology findings

The median goblet cell density was significantly lower in the AKC group (116 cells/mm² [IQR: 58–289]) compared with the control group (463 cells/mm² [IQR: 123–1063]; $p<0.001$), representing a 75% reduction in goblet cell density. The median Nelson score was significantly higher in the AKC group (2.0 [IQR: 1.0–2.0]) compared with controls (1.0 [IQR: 0.0–1.75]; $p<0.001$), indicating more advanced squamous metaplasia in the post-AKC eyes (Fig. 2).

In the AKC group, Nelson grade 2 was the most common finding (48.4%), followed by grade 1 (31.6%), grade 3 (14.7%), and grade 0 (5.3%). In contrast, the control group showed predominantly grade 0 (38.0%) and grade 1 (42.4%) findings, with fewer eyes showing grade 2 (17.4%) or grade 3 (2.2%) (Fig. 3).

Artificial tear subgroup analysis

A subgroup analysis within the AKC group comparing patients using preservative-free artificial tears ($n=42$) with those not using them ($n=22$) revealed no statistically significant differences in goblet cell density (median: 116 [IQR: 58–260] vs. 145 [IQR: 58–347] cells/mm²; $p=0.412$) or TBUT (7.89 ± 2.85 vs. 8.67 ± 3.18 s; $p=0.287$), suggesting that artificial tear use did not significantly confound the observed between-group differences.

Sex-stratified and multivariable analysis

Given the significantly higher proportion of females in the AKC group, sex-stratified subgroup analyses were performed. In male participants, goblet cell density was significantly lower in the AKC group compared with controls (median: 145 [IQR: 58–318] vs. 434 [IQR:

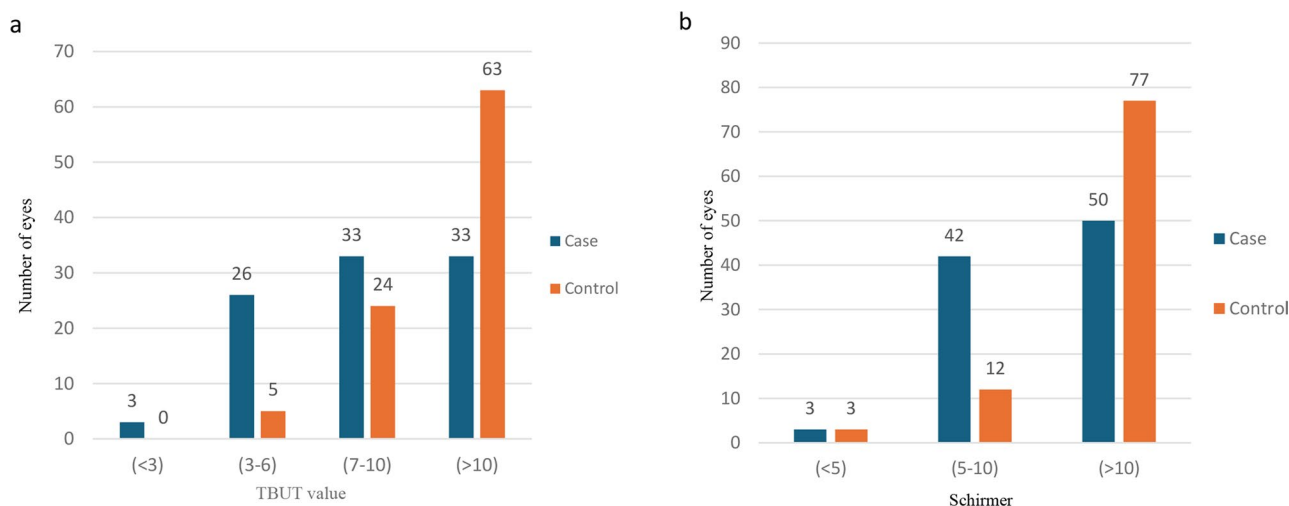


Fig. 1 **a** Distribution of tear breakup time (TBUT) values in the AKC case group and healthy control group. TBUT values are categorised as: <3 s, 3–6 s, 7–10 s, and >10 s. In the AKC group, 67.4% of eyes (64/95) had TBUT values below 10 s, compared with 30.4% in the control group. **b** Distribution of Schirmer test values (with anaesthesia) in the AKC case group and healthy control group. Values are categorised as: <5 mm, 5–10 mm, and >10 mm. In the AKC group, 52.6% of eyes (50/95) had Schirmer values below 10 mm, compared with 16.3% in the control group

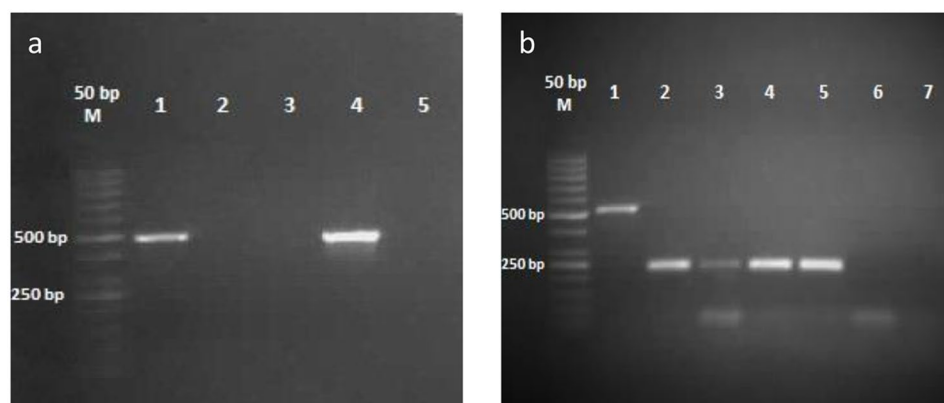


Fig. 2 Adenovirus PCR detection and serotyping results. **(a)** Adenovirus DNA detection by PCR targeting the hexon gene conserved region. Lane 1: positive patient (band present); Lane 2–3: negative patients; Lane 4: adenovirus positive control (475 bp); Lane 5: adenovirus negative control. **(b)** Serotyping by multiplex PCR. Lane 1: serotype 3 (517 bp); Lanes 2–5: serotype 8 (243 bp); Lane 6: negative control; Lane 7: mixture control; M: 50 bp DNA ladder

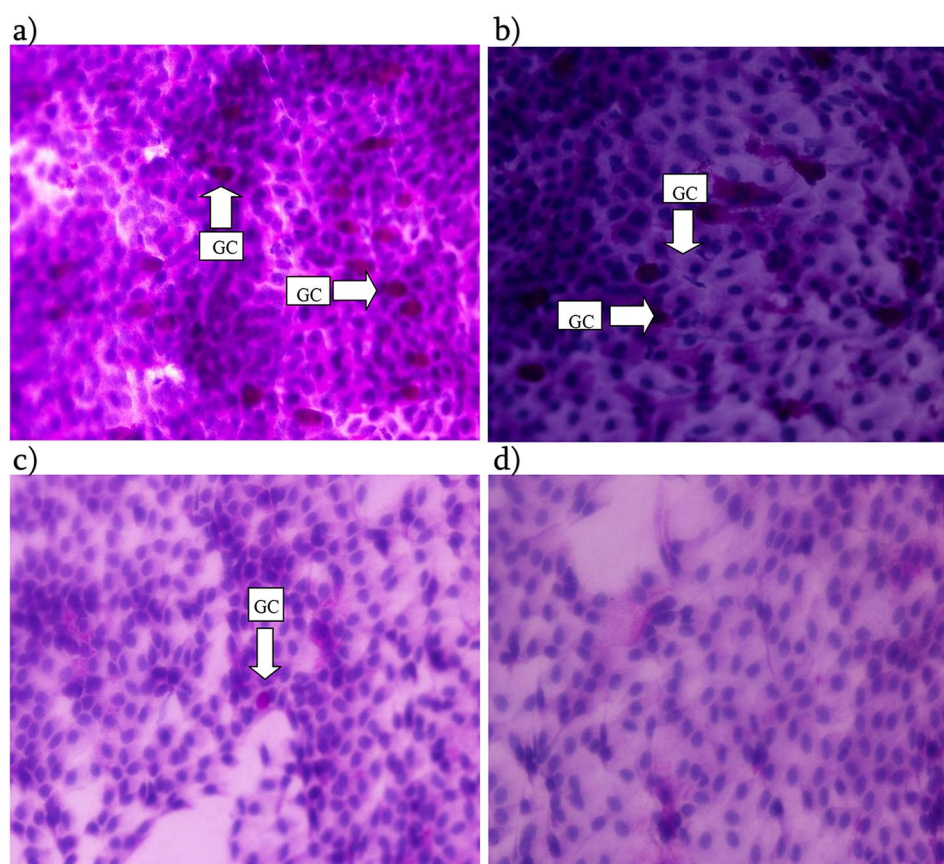


Fig. 3 Conjunctival impression cytology images (periodic acid-Schiff staining, x400 magnification). **(a)** Representative image from a healthy control eye showing abundant, well-preserved goblet cells (GC, arrows) with characteristic PAS-positive cytoplasm and small, round nuclei consistent with Nelson grade 0–1. **(b)** Second representative control image demonstrating multiple goblet cells with preserved oval morphology. **(c)** Representative image from a post-AKC eye showing Nelson grade 2 changes: markedly reduced goblet cell density, enlarged polygonal epithelial cells with small nuclei, and scattered residual goblet cells. **(d)** Representative image from a post-AKC eye showing Nelson grade 3 changes: complete absence of goblet cells, large polygonal cells with pyknotic nuclei, and advanced squamous metaplasia

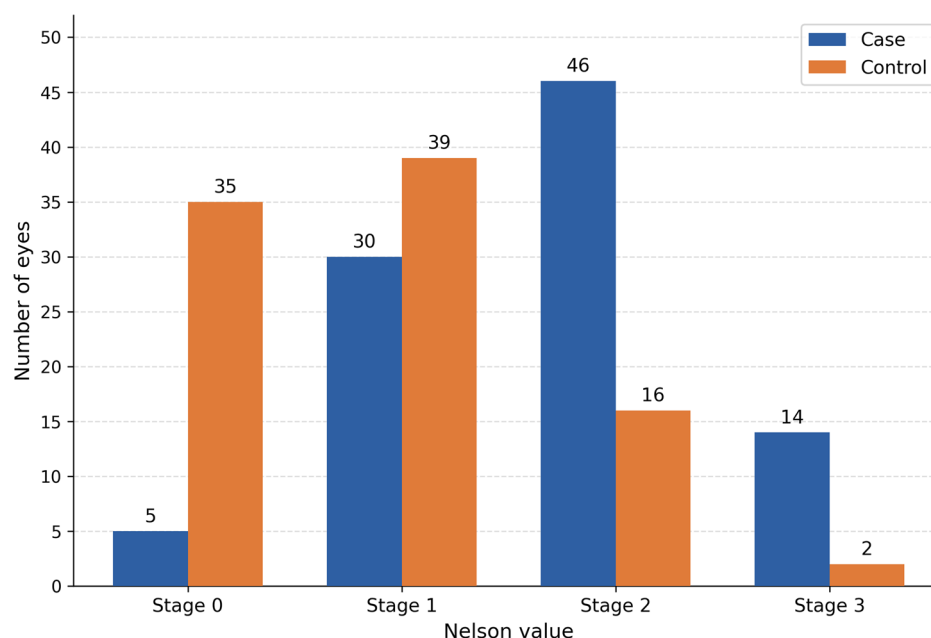


Fig. 4 Distribution of Nelson classification scores in the AKC case group ($n=95$ eyes) and healthy control group ($n=92$ eyes). AKC eyes showed predominantly Nelson grade 2 changes ($n=46$, 48.4%), followed by grade 1 ($n=30$, 31.6%), grade 3 ($n=14$, 14.7%), and grade 0 ($n=5$, 5.3%). Control eyes showed predominantly grade 1 ($n=39$, 42.4%) and grade 0 ($n=35$, 38.0%), with fewer grade 2 ($n=16$, 17.4%) or grade 3 ($n=2$, 2.2%)

116–1013] cells/mm²; $p<0.001$). Similarly, in female participants, goblet cell density remained significantly reduced in the AKC group (median: 116 [IQR: 58–275] vs. 492 [IQR: 145–1100] cells/mm²; $p<0.001$). TBUT and Schirmer test values followed the same pattern in both sex-specific subgroups, with all between-group comparisons reaching statistical significance ($p<0.001$ for all).

Binary logistic regression analysis confirmed that AKC group membership was a significant independent predictor of low goblet cell density after adjusting for sex and age (adjusted odds ratio: 4.82; 95% CI: 2.61–8.91; $p<0.001$). Neither sex (adjusted OR: 1.34; 95% CI: 0.84–2.15; $p=0.218$) nor age (adjusted OR: 1.01; 95% CI: 0.99–1.03; $p=0.341$) was a statistically significant independent predictor, confirming that the observed goblet cell reduction is attributable to AKC history rather than sex imbalance or age differences.

Adenovirus serotyping

Among the six patients who underwent virological testing during the acute phase, adenovirus DNA was detected in five patients (83.3%). Serotyping revealed adenovirus serotype 8 in four patients (80%) and serotype 3 in one patient (20%). The patient with negative PCR result was excluded from subsequent analyses (Fig. 4).

Discussion

This study demonstrates that AKC leads to significant long-term alterations in the conjunctival epithelium and tear film parameters. At a mean follow-up of 18 months after the acute infection, patients with a history of AKC showed a 75% reduction in goblet cell density, increased squamous metaplasia, and decreased tear film stability compared with healthy controls. These findings support our hypothesis that AKC causes persistent damage to the ocular surface, contributing to chronic dry eye disease. The finding that ocular and systemic infections may trigger persistent dry eye disease is increasingly recognised; a recent systematic review by Kothandan et al. specifically examined the characteristics of dry eye disease secondary to ocular and systemic infections, identifying post-infectious DED as a distinct and underappreciated aetiological category [24]. The present study provides prospective case-control evidence for adenoviral keratoconjunctivitis as a contributor to this entity, quantifying the magnitude of goblet cell loss and tear film destabilisation at a mean of 18 months post-infection.

Conjunctival goblet cells are essential for maintaining ocular surface homeostasis through the secretion of gel-forming mucins, primarily MUC5AC [8]. These mucins form the mucous layer of the tear film, which lubricates the ocular surface and provides protection against pathogens and debris [25]. Dogru et al. [26] reported significantly lower levels of MUC5AC in the tears of dry eye patients compared with healthy individuals, suggesting

that goblet cell dysfunction plays a central role in dry eye pathophysiology. Our finding of markedly reduced goblet cell density in post-AKC patients is consistent with these observations and provides a mechanistic explanation for the dry eye symptoms experienced by these patients.

The TBUT is a widely used clinical parameter for assessing tear film stability and is particularly sensitive to deficiencies in the mucin and lipid components of the tear film [5]. In our study, the mean TBUT was significantly lower in the AKC group (8.16 s) compared with controls (11.1 s), with 67.4% of post-AKC eyes showing TBUT values below the normal threshold of 10 s. The reduced TBUT in conjunction with decreased goblet cell density suggests that mucin deficiency is a major contributor to tear film instability in these patients.

Our serotyping results identified adenovirus serotype 8 as the predominant strain, consistent with its well-documented association with EKC worldwide [3, 27]. Serotype 8 is known for its enhanced tropism for conjunctival epithelial cells and its ability to cause severe ocular surface inflammation [27]. The predominance of this serotype in our cohort may partly explain the significant long-term ocular surface changes observed, as more virulent strains might cause greater tissue damage during the acute phase.

We acknowledge that the clinical diagnosis of AKC without universal PCR confirmation represents an inherent limitation. However, several factors support the accuracy of our diagnostic approach. First, all cases were identified during well-documented epidemic outbreaks at our institution, where the clinical presentation of EKC was highly characteristic; epidemic clustering significantly increases the positive predictive value of clinical diagnosis for adenoviral etiology [1]. Second, strict exclusion criteria were applied to minimize misclassification with bacterial, allergic, or other viral etiologies. Third, PCR confirmation in a subset of patients yielded an 83.3% positivity rate with predominant serotype 8, providing supportive evidence for the accuracy of our clinical diagnostic approach. It should be noted that in clinical practice, the vast majority of AKC cases are managed based on clinical diagnosis alone, as point-of-care antigen detection tests have reported sensitivities ranging from 39% to 89% depending on the test generation and clinical setting [14, 15], and PCR-based testing is not routinely available in most settings, particularly during epidemic surges. The single PCR-negative result in the virological subset is noteworthy; this patient's swab was collected on day 10 of symptom onset, at which timepoint viral shedding is known to decrease substantially, likely accounting for the false-negative result.

The higher proportion of females in the AKC group (64.1%) compared with the control group (41.8%) warrants discussion, as sex hormones are known to influence

tear film composition and ocular surface health [28]. However, our sex-stratified subgroup analysis demonstrated that the significant reduction in goblet cell density and tear film parameters in the AKC group was consistent across both sexes. Furthermore, multivariable logistic regression analysis confirmed that AKC group membership remained a significant independent predictor of low goblet cell density after adjusting for sex and age, while sex itself was not a significant predictor. The female predominance in our AKC cohort is consistent with the reported epidemiology of adenoviral conjunctivitis, where women are disproportionately represented, potentially due to greater exposure through healthcare, educational, and caregiving roles.

Several mechanisms may explain the persistent goblet cell loss following AKC. First, the intense inflammatory response during the acute phase likely causes direct cytotoxic damage to goblet cells [29]. Second, chronic low-grade inflammation may persist even after clinical resolution, leading to progressive goblet cell depletion [30]. Third, squamous metaplasia, once established, may be irreversible in some patients, perpetuating the ocular surface dysfunction [31]. The elevated Nelson scores observed in our AKC group support the presence of advanced squamous metaplasia, which may represent a form of permanent ocular surface damage.

An important clinical observation in this cohort is the potential dissociation between objective tear film changes and subjective symptoms in post-AKC patients. Objective tear film parameters and goblet cell density may be significantly altered in patients who do not spontaneously report dry eye symptoms, particularly at intermediate follow-up timepoints [32]. This phenomenon of subclinical ocular surface disease following viral conjunctivitis may be partly attributable to concurrent neurosensory changes — including reduced corneal sensitivity following adenoviral epithelial damage — that blunt the afferent arc of the symptom-reporting pathway [19, 33]. The clinical implication is that reliance on patient-reported symptoms alone will substantially underestimate the true burden of ocular surface disease in the post-AKC population. Objective screening with tear film assessment and impression cytology, irrespective of symptom status, is therefore warranted at all follow-up visits, and clinicians should maintain a low threshold for intervention in patients with abnormal objective parameters even in the absence of overt dry eye complaints.

Our findings have important clinical implications. First, patients recovering from AKC should be counseled about the potential for long-term dry eye symptoms and the need for continued follow-up. Second, early and aggressive anti-inflammatory treatment during the acute phase might potentially reduce the degree of goblet cell loss and subsequent dry eye development, although this

hypothesis requires further investigation. Third, long-term management strategies, including artificial tears, anti-inflammatory agents, and mucin secretagogues, may be necessary in patients with persistent symptoms.

Limitations

This study has several limitations. First, AKC diagnosis was based primarily on clinical criteria without universal PCR confirmation; although PCR validation in a subset (83.3% positivity) and the epidemic context support the accuracy of our approach, some degree of diagnostic misclassification cannot be excluded. Second, a priori sample size calculation was not performed; however, post-hoc power analysis confirmed that the study was adequately powered (>99%) for the primary outcome. Third, we did not have baseline impression cytology data from the acute phase, which would have allowed direct quantification of goblet cell loss over time. Fourth, tear osmolarity measurement and formal meibography were not performed; these parameters were not available at our institution during the study period, and their absence limits the comprehensiveness of the ocular surface evaluation. Fifth, although patients with clinical meibomian gland dysfunction were excluded based on slit-lamp evaluation and manual gland expression, subclinical MGD may have been present in some participants. Sixth, the significantly higher proportion of females in the AKC group was addressed through sex-stratified analysis and multivariable regression, both of which confirmed that AKC remained the primary determinant of goblet cell loss. Seventh, while all topical anti-inflammatory agents were excluded, the higher rate of artificial tear use in the AKC group represents a potential confounder; however, subgroup analysis showed no significant effect of artificial tear use on the primary outcomes, and any potential bias from artificial tears would favor conservative estimates of the true between-group difference. Eighth, adenovirus serotyping was performed in only a small subset of patients, limiting the ability to correlate specific serotypes with clinical outcomes. Ninth, the study was conducted at a single center, which may limit generalizability. Tenth, potential confounders such as smoking status, menopausal status, and environmental exposure factors were not systematically recorded; although these variables may influence goblet cell density and tear film parameters, they were not available for adjustment in the multivariable analysis. Furthermore, a formal dry eye symptomatology assessment using a validated questionnaire such as the Ocular Surface Disease Index (OSDI) was not systematically performed in this study. This represents a limitation, as patient-reported outcomes provide important complementary information to objective tear film measurements, particularly for characterising the symptomatic burden of post-AKC ocular surface

disease and identifying the subgroup of patients with objective changes but absent or attenuated symptoms. Finally, the cross-sectional nature of the assessment precludes evaluation of the temporal progression of ocular surface changes following AKC.

Conclusions

This study demonstrates that AKC leads to significant and persistent changes in the conjunctival epithelium, characterized by reduced goblet cell density and squamous metaplasia, which are associated with decreased tear film stability at 18 months post-infection, independent of sex and age. These findings highlight the importance of long-term follow-up and appropriate dry eye management in patients recovering from AKC. Future prospective studies with serial evaluations from the acute phase through recovery are needed to fully characterize the natural history of ocular surface changes following adenoviral infection and to identify strategies for preventing chronic dry eye in this population.

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Author contributions

MMT and VLK conceived and designed the study. MMT and DKY collected the data and performed the clinical examinations. MMT analyzed the data and drafted the manuscript. FS, EOT, and AS critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Kocaeli University Faculty of Medicine (Decision No: 2016/13.9). All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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