



# Conjunctival Impression Cytology and Tear Function in Patients with Keratoconus

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## Abstract

**Objectives:** The aim of this study was to evaluate the ocular surface alterations of conjunctival cytological changes and tear function in patients with keratoconus.

**Methods:** A total of 98 eyes with keratoconus and 68 eyes of age- and sex- matched healthy subjects were included in this prospective study. Ophthalmological evaluations, including Ocular Surface Disease Index (OSDI), tear break-up time (TBUT), ocular surface staining (OSS), Schirmer test, and conjunctival impression cytology (CIC), were performed.

**Results:** The mean participant age was 23.4±5.3 years in the keratoconus group and 24.9±6.0 years in the control group. In the keratoconus group, the mean OSDI and OSS scores were statistically significantly higher than those of the controls, and the Schirmer and TBUT results were statistically significantly lower (all  $p < 0.001$ ). The severity of keratoconus was positively correlated with the CIC grade ( $r = 0.292$ ;  $p = 0.004$ ).

**Conclusion:** Keratoconus results in deterioration of the ocular surface, including CIC and tear function parameters. The grade of keratoconus appears to be associated with conjunctival cytologic changes.

**Keywords:** Conjunctival impression cytology, keratoconus, ocular surface, tear functions

## Introduction

Keratoconus is a progressive corneal ectasia that can lead to irregular astigmatism and loss of vision. The disease frequently begins during puberty; a reported incidence in the population is 1 in 375 between the ages of 10-40 years (1). Although the etiology of keratoconus remains unclear, a relationship between keratoconus and ocular surface disorders, such as allergies, corneal sensitivity, and tear film changes, has been reported in previous studies (2,3).

Conjunctival impression cytology (CIC) is a minimally invasive technique used to evaluate ocular surface cell mor-

phology. It can help to identify a variety of ocular diseases, demonstrate the effects of treatments, and quantify inflammatory biomarkers (4,5).

In clinical experience, ocular surface complaints are frequently seen in keratoconus patients in addition to visual symptoms. The hypothesis of this research was that diagnostic dry eye disease (DED) tests could reveal pathological changes on the ocular surface of keratoconus patients. This study is an analysis of DED tests used to evaluate conjunctival cytological changes and tear function in patients with keratoconus. The findings may help to explain the relationship between keratoconus and ocular surface alterations.

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## Methods

The study protocol was approved by the Hatay Mustafa Kemal University Tayfur Ata Sokmen Faculty of Medicine clinical research ethics committee (No: 2020/34, Date: 20/02/2020). The research was conducted in accordance with the ethical standards of Helsinki Declaration. All of the participants provided a written, informed consent form.

This prospective, case-controlled study was performed at the ophthalmology and pathology clinics of a university hospital. In all, 98 patients with keratoconus and 68 age- and sex- matched healthy subjects were enrolled.

The diagnosis of keratoconus was made based corneal thinning and ectasia findings detected in a clinical examination and corneal topography using a Sirius device (Costruzioni Strumenti Oftalmici SRL, Florence, Italy). The severity of keratoconus was determined according to the Amsler-Krumeich keratoconus classification (6). The control group consisted of participants who presented for a refraction examination and had 20/20 visual acuity. Only 1 eye of each participant was enrolled in the study. Patients with advanced keratoconus (grade 4) and those who had a history of ocular trauma or surgery, continuous use of ocular medication or contact lenses, or any ocular and systemic disorder that could affect the anterior surface of the eye were excluded from the study.

Ophthalmic evaluations, including the Ocular Surface Disease Index (OSDI; Allergan Inc., Irvine, CA, USA) questionnaire, tear break-up time (TBUT) measurement, ocular surface staining (OSS), the Schirmer I test, and CIC were performed for all of the study participants. The OSDI questionnaire was used to evaluate subjective levels of ocular discomfort and vision-related dysfunction in the previous 2 weeks (7). Subsequently, fluorescein was administered to the ocular surface and the patient was asked to blink naturally 3 times. TBUT was determined by measuring the interval between the instillation of topical fluorescein and the appearance of the first dry spots on the corneal tear film. Corneal and conjunctival staining were evaluated using fluorescein and Lissamine green dye (MilliporeSigma, Burlington,

MA, USA) Grading the severity of the OSS was performed according to a previously reported ocular staining grading system (8). The Schirmer I test was performed without anesthesia by measuring wetness 5 minutes after applying a paper strip (5x35 mm) on the temporal one-third of the lower lid margin (9).

CIC was performed as follows: Following the administration of topical anesthesia with proparacaine, a cellulose acetate filter strip (3x5 mm, pore size: 0.2  $\mu$ m; Sartorius Stedim Biotech GmbH, Göttingen, Germany) was placed on the temporal bulbar conjunctiva for 10 seconds. The strip was then fixed in 95% ethyl alcohol solution for 2 hours and subsequently stained with periodic acid-Schiff in the pathology department. All of the conjunctiva specimens collected were evaluated by a single pathologist (DG) who was unaware of the clinical details. Nelson's grading schema (grade 0-3) was used to categorize squamous metaplasia according to a microscopy evaluation (10, 11). The grade 0 and grade 1 nucleus/cytoplasm ratio is 1:2 to 1:3, and grade 2 and grade 3 have a ratio of 1:4 to 1:6.

## Statistical Analysis

All of the statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 21.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as number and percentage, and quantitative variables were described using mean $\pm$ SD. The normality of the distribution of the variables was assessed using the Kolmogorov-Smirnov test. According to the data distribution, the Student t-test or the Mann-Whitney U test was used to determine significant differences between study groups. Correlations between the keratoconus stage and ocular surface parameters were analyzed with Spearman or Pearson correlation. The level of statistical significance was set at  $p < 0.05$ .

## Results

The demographic details of the study groups are summarized in Table 1. In the keratoconus group, the mean age was 23.4 $\pm$ 5.3 years and 45.9% of the patients were female. The mean age was 24.9 $\pm$ 6.0 years and 44.1% of the participants

**Table 1.** Demographic details of the study groups

	Keratoconus patients (n=98)	Healthy controls (n=68)	p
Age (years)			
Mean $\pm$ SD (min-max)	23.4 $\pm$ 5.3 (16-37)	24.9 $\pm$ 6.0 (17-44)	0.092*
Gender (n)			
Female	45	30	
Male	53	38	0.819**

\*: Student's t-test; \*\*: Pearson chi-squared test.

were female in the control group. Table 2 illustrates the ocular surface parameters of the study groups. The OSDI, Schirmer test, TBUT, OSS scores, and CIC grades were clinically poorer in the keratoconus group compared with the healthy subjects. The mean OSDI ( $35.49 \pm 12.1$  vs  $21.6 \pm 17.6$ ) and OSS ( $1.94 \pm 1.3$  vs  $1.02 \pm 0.9$ ) scores were statistically significantly higher and the Schirmer ( $4.32 \pm 2.6$  vs  $12.56 \pm 3.8$ ) and TBUT ( $9.2 \pm 2.9$  vs  $13.4 \pm 4.5$ ) values were statistically significantly lower in the keratoconus group than those of the controls (all  $p < 0.001$ ). The CIC classification was grade 0 or 1 in 70% and grade 2 or 3 in 30% of the keratoconus group. In the control group, 91% were grade 0 or 1 and 9% were categorized as grade 2 or 3.

Of the 98 keratoconic eyes, 42.9% eyes were classified as grade 1 keratoconus, 33.7% were grade 2, and 22.4% were grade 3. Analysis of the correlations between the keratoconus grade and ocular surface parameters revealed a significant positive correlation between the keratoconus grade and the CIC grade (Spearman correlation coefficient value [95%]: 0.292; all  $p = 0.004$ ) (Table 3).

## Discussion

In the present study, we investigated ocular surface alterations in patients with keratoconus and the relationship to the severity of the disease using diagnostic DED tests. The results of this research showed that patients with keratoconus had statistically significantly higher OSDI scores, reduced tear volumes, increased tear instability, greater ocular surface staining, and higher CIC grades than healthy controls. In addition, a statistically significantly positive correlation was observed between keratoconus and the CIC grade.

The degradation of corneal layers has a key role in the development of keratoconus (12). Collagen degradation products released into tears adversely affect the stability of the tear film (3). Furthermore, both stromal and epithelial thinning occur during the progression of keratoconus (13). Recent research has examined the role of inflammatory processes in the pathophysiology of keratoconus. Increased levels of inflammatory molecules, such as interleukin-6, tumor necrosis factor-alpha, and matrix metalloproteinase 9, have been demonstrated in keratoconus eyes in several studies

**Table 2.** Comparison of the ocular surface parameters of the study groups

	Keratoconus patients (n=98)	Healthy controls (n=68)	p
OSDI score (Mean±SD)	35.49±12.1	21.6±17.6	<0.001*
Schirmer test (mm) (Mean±SD)	4.32±2.6	12.56±3.8	<0.001**
TBUT (s) (Mean±SD)	9.2±2.9	13.4±4.5	<0.001**
OSS score (Mean±SD)	1.94±1.3	1.02±0.9	<0.001**
CIC grade (n, %)			<0.001***
Grade 0	25 (25.5)	55 (80.9)	
Grade 1	44 (44.9)	7 (10.3)	
Grade 2	20 (20.4)	5 (7.4)	
Grade 3	9 (9.2)	1 (1.5)	

\*: Student's t-test; \*\*: Mann-Whitney U test; \*\*\*: Pearson chi-squared test; CIC: Conjunctival impression cytology; OSDI: Ocular Surface Disease Index; OSS: Ocular surface staining; TBUT: Tear break-up time.

**Table 3.** Correlation between keratoconus grade and ocular surface parameters

	OSDI score	Schirmer test	TBUT	OSS score	CIC grade
Keratoconus grade					
r	0.085	-0.049	-0.112	0.107	0.292
p	0.406*	0.586**	0.271**	0.296**	0.004**
n	98	98	98	98	98

\*: Pearson's correlation analysis; \*\*: Spearman's correlation analysis; CIC: Conjunctival impression cytology; OSDI: Ocular Surface Disease Index; OSS: Ocular surface staining; TBUT: Tear break-up time.

(14, 15). Corneal trauma due to a contact lens or eye rubbing can provoke a strong inflammatory response in the corneal epithelium in patients with keratoconus (16, 17).

Higher OSDI and OSS scores, lower Schirmer I test results, and comparable TBUT measurements have been reported in keratoconus patients and healthy subjects (18). Moreover, patients with keratoconus have been observed to have a statistically significantly lower mucin cloud height and goblet cell layer thickness values and higher tear concentrations of di-adenosine tetraphosphate than healthy subjects. In our study, the TBUT measurements were statistically significantly lower in keratoconus patients, and the CIC findings were found to be positively correlated with the keratoconus grade using the Nelson grading schema. Other researchers also found lower TBUT measurements and higher OSS scores and CIC grades in patients with keratoconus compared with controls (3). As the keratoconus stage increased, the authors of that study found that the results of these measurements worsened. In contrast we only found a statistically significant correlation between the CIC grade and the keratoconus stage.

De Paiva et al. (19) reported that surface regularity indices, which are elevated in patients with DED, can be used to diagnose DED and predict the severity of the disease. Decreased TBUT measurements as a result of topographic steepening of the cornea in cases of keratoconus have been observed (3). However, another study evaluating the association between topographic/tomographic values and tear film parameters in keratoconus patients reported no interaction, as we also observed (20).

Our findings revealed significant levels of conjunctival squamous metaplasia and loss of goblet cells in the keratoconus eyes. There was a significant positive correlation between the keratoconus stage and the CIC grade. This may be related to the epithelial origin of the disease and the relationship between keratoconus and inflammation.

Limitations of the present study include the single-center design, a relatively small number of patients, and a lack of corneal sensitivity measurements and biomarkers to provide more evidence about tear components.

The results of the current study showed that the patients with keratoconus had clinically poorer diagnostic DED measurements than the healthy subjects. Our findings support the concept that adverse changes in the ocular surface in cases of keratoconus affect not only the cornea, but the tear film and conjunctival epithelium as well. In addition, the CIC grade was found to correlate with the stage of keratoconus. Therefore, CIC assessment may provide useful information to understanding the ocular surface alterations of patients with keratoconus. Our results should be confirmed by additional studies investigating the relationship between keratoconus and ocular surface parameters.

## Disclosures

**Ethics Committee Approval:** Hatay Mustafa Kemal University Tayfur Ata Sokmen Faculty of Medicine Clinical Research Ethics Committee, protocol number: 2020/34, Date: 20/02/2020.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship Contributions:** Involved in design and conduct of the study (DOO, SCO, DG); preparation and review of the study (DOO, SCO); data collection (DOO, SCO, DG); and statistical analysis (DOO, SCO).

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