Dry eye disease and ocular surface characteristics in patients with keratoconus

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Abstract:

PURPOSE: The purpose of this study is to investigate the ocular surface alterations in patients with mild or severe keratoconus (KC).

METHODS: A total of 80 participants were included in the study. The corneal topography was performed on each participant using Pentacam and the grouping was done accordingly. The patients with Kmax ≥52.0 D (severe KC) were considered Group 1 (n = 28), the patients with Kmax ≥47.2 and <52.0 D (mild KC) were considered Group 2 (n = 30). Healthy control participants with Kmax <47.2 D were considered Group 3 (n = 22). Tear breakup time (TBUT), Schirmer-I test, ocular surface disease index (OSDI) questionnaire, and conjunctival impression cytology (CIC) were evaluated among the groups.

RESULTS: The mean values of TBUT and Schirmer-I test were significantly lower (P = 0.012, P = 0.012) and the mean scores of OSDI and CIC were significantly higher (P = 0.006, P < 0.001) in Group 1 and Group 2 than in Group 3. The mean values of TBUT and Schirmer-I test were lower and the mean scores of OSDI and CIC were higher in Group 1 than in Group 2 but the differences were insignificant (P > 0.05 for all).

CONCLUSION: These results indicated that the tests associated with dry eye disease are correlated with KC. Tear film alterations and goblet cell loss are higher in severe KC.

Keywords:

Conjunctival impression cytology, dry eye disease, goblet cell density, keratoconus, tear film

Introduction

Bilateral, asymmetric, progressive, and noninflammatory corneal ectatic disorder which leads to visual distortion, low vision, and even blindness due to an abnormal structure of the cornea is the classical definition of keratoconus (KC).[1]

The prevalence of KC is approximately 0.2%–5.4% of the population and the presentation of this corneal disease is usually at the second or third decades of life.[1,2] The cause of KC remains unclear but it includes several factors such as systemic diseases, syndromes, allergy, ultraviolet light exposure, genetic inheritance, and eye rubbing.[3]

With an increased comprehension of this disease, despite the classical knowledge, it has been understood that inflammation may play an important role in the development of KC.[4] Clinical findings of inflammation such as pain, heat, redness, and edema are not observed in KC eyes but recent studies have shown that inflammation mediators in the tear and in the ocular surface are significantly higher and anti-inflammatory mediators are lower than in healthy eyes.[5,6] Indeed, it is demonstrated that there is a relationship between KC and dry eye disease (DED).[7,8]

For this reason, investigating the changes in the tear film and the conjunctival cells may elucidate the pathophysiological mechanisms of KC. Conjunctival impression cytology (CIC) is a diagnostic test for DED and squamous metaplasia in ocular surface disease.[9,10] CIC is a minimally invasive biopsy method of obtaining specimens from the conjunctiva and assessing the density of conjunctival goblet cells.

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In brief, very few studies have investigated the association between DED and KC and performed CIC to correlate these diseases. Thus, the objective of this study was to evaluate the relationship between ocular surface alterations and the severity of KC.

Methods

Fifty-eight contact lens–naïve KC patients which were newly diagnosed as KC and 22 control subjects were enrolled in this prospective study. All participants were recruited from the Ophthalmology Department at Meram Faculty of Medicine, Necmettin Erbakan University between June 2017 and June 2018. The study was approved by the local ethics committee and followed the tenets of the Declaration of Helsinki (No: 2017/1091).

Both eyes of all participants underwent a comprehensive ophthalmic assessment including best-corrected visual acuity, slit-lamp biomicroscopy, fundus examination, and corneal topography. Corneal topography was performed with a rotating Scheimpflug camera (Pentacam HR, Oculus Optikgerate, Wetzlar, Germany) by the same technician. KC was diagnosed due to corneal topographic results according to the guidelines provided by Rabinowitz (K value >47.2 D and/or inferior–superior value of >1.4 D).[11] The eyes of patients with KC were divided into two groups based on Kmax readings. The eye with higher Kmax value in KC patients and one randomized eye of control subjects were included in the study. Twenty-eight eyes of 28 KC patients with Kmax ≥52.0 D (severe) were regarded as Group 1, 30 eyes of 30 KC patients with Kmax ≥47.2 or <52.0 D (mild) were regarded as Group 2 and compared with 22 eyes of 22 control subjects with Kmax <47.2 D (Group 3).

All study participants had never worn contact lenses. At the time of this study, no participant was being treated with topical eye medications or systemic medications. KC patients with systemic diseases, history of previous any ocular surgery, chemical or thermal burns were not included in the study. Control subjects who presented abnormal topographic patterns, already diagnosed with DED or systemic diseases were not included, either.

Tear breakup time (TBUT), Schirmer-I test, and CIC were performed by the same researcher. A sterile paper containing fluorescein sodium was applied to the inferior conjunctival sac and a cobalt blue filter on slit-lamp examination was used for determining measurements of TBUT (Fluorescein paper; Haag-Streit AG, Köniz, Switzerland). The interval between the last complete blink and the consecutive first dry spot was regarded as the value of TBUT. After an average of three measurements, a value of <10 s (sec) was considered an abnormal TBUT. The Standard Whatman filter paper strips (Whatman, Maidstone, United Kingdom) were placed on the outer third part of the lower eyelid for Schirmer-I test. The average length of wet strips with no anesthetic after 5 min was noted in millimeters (mm) as the value of Schirmer-I test. The next day after TBUT measurements and Schirmer-I test, CIC test was done. The nitrocellulose acetate filter papers (Sartorius AG, Goettingen, Germany) were pressed on the inferior temporal bulbar conjunctiva, 3 mm away from the limbus for collecting CIC samples. The samples were evaluated and graded by one of the authors (PO) using the criteria suggested by Nelson (Grade 0: greater than 500 goblet cells/mm^2, small, round epithelial cells with large nuclei; grade 1 to 2: 100 to 500 goblet cells/mm^2; grade 3: less than 100 goblet cells/mm^2, large, polygonal epithelial cells with small nuclei [Figures 1 and 2]).[12] After that, all participants completed the Ocular Surface Disease Index (OSDI) questionnaire which is used to grade the stage of DED symptoms. The questionnaire included 12 questions and all questions have five possible responses from 0 to 4 (0 = none of the time, 1 = some of the time, 2 = half of the time, 3 = most of the time, and 4 = all of the time). The questionnaire has scores ranged 0–100 and the patients who have an advanced degree of DED symptoms response higher scores. After that, all participants completed the OSDI questionnaire which is used to grade the stage of DED symptoms. The questionnaire included 12 questions and all questions have five possible responses from 0 to 4 (0 = none of the time, 1 = some of the time, 2 = half of the time, 3 = most of the time, and 4 = all of the time). The questionnaire has scores ranged 0–100 and the patients who have an advanced degree of DED symptoms response higher scores.

SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive analyses were presented using mean values and standard deviations for age, corneal topography parameters, TBUT values, Schirmer-I test values, OSDI, and CIC scores. The Kolmogorov–Smirnov test was used to test the normality of distribution. Statistical significance was determined

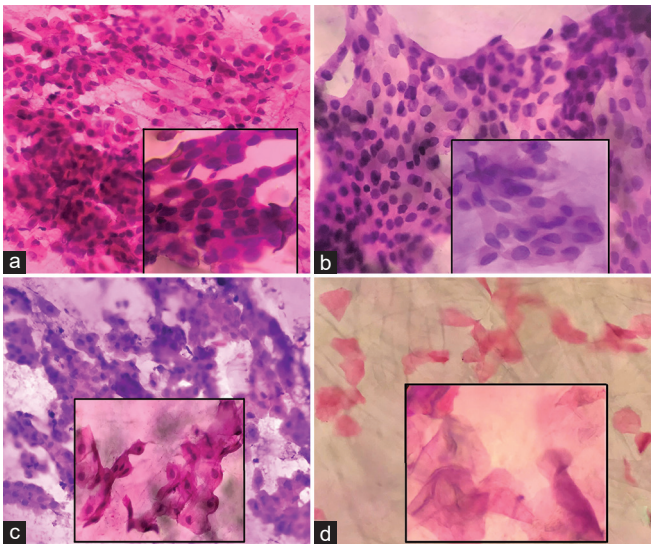

Figure 1: (a) CIC Grade 0 (b) CIC Grade 1 (c) CIC Grade 2 (d) CIC Grade 3 (All the big pictures, H and E ×400; all the small pictures, H and E, ×100). CIC: Conjunctival impression cytology

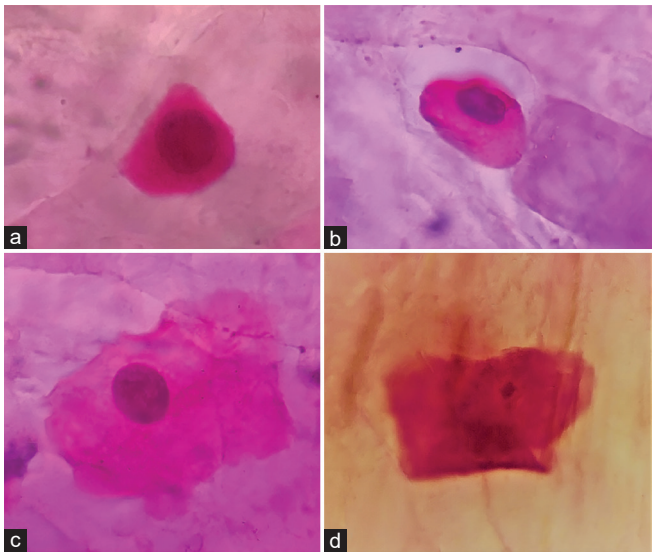

Figure 2: H and E squamous cell morphologies according to the Nelson criteria (All the squamous cells in H and E stain of impression cytology have dens pink cytoplasm); (a) Grade 0; NCR 1:2 (b) Grade 1; NCR 1:3 (c) Grade 2; NCR 1:4 (d) Grade 3; Pyknotic nucleus in large cytoplasm (×1000)

by nonparametric Mann–Whitney *U*-tests. $P < 0.05$ was considered statistically significant.

Results

The age of participants ranged between 18 and 54-year-old and the mean age of the participants was 27.8 ± 9.5 years in Group 1, 26.9 ± 8.5 years in Group 2, and 28 ± 9.4 years in Group 3. The demographic features such as age and gender were similar among the three groups [$P = 0.910$, $P > 0.05$, Table 1].

The corneal topography parameters, tear function test results and CIC scores were summarized in Table 1. The mean Kmax values were significantly higher in Group 1 (59.5 ± 8.7 D) and Group 2 (49.5 ± 1.8 D) than in Group 3 (44.8 ± 1.2 D, $P < 0.001$). The mean thinnest values were significantly lower in Group 1 (428.5 ± 46.7 μm) and Group 2 (473.9 ± 32.5 μm) than in Group 3 (523.6 ± 35.3 μm, $P < 0.001$).

The mean TBUT values were significantly lower in Group 1 (6.9 ± 3.1 s) and Group 2 (7.3 ± 3 s) than in Group 3 (11.8 ± 1.6 s, $P = 0.012$). The mean Schirmer-I test values were significantly lower in Group 1 (13.3 ± 8 mm) and Group 2 (16.6 ± 9.2 mm) than in Group 3 (21.7 ± 9.1 mm, $P = 0.012$). The OSDI questionnaire scores were significantly higher in Group 1 (41.1 ± 25.7) and Group 2 (35.3 ± 23.6) than in Group 3 (19.1 ± 10.2, $P = 0.006$).

The CIC scores were significantly higher in Group 1 (1.9 ± 0.8) and Group 2 (1.6 ± 0.7) than in Group 3 (0.6 ± 0.0.5, $P < 0.001$). Notably, none of the patients in Group 1 showed Grade 0 differentiation and none of the patients in Group 3 showed Grade 2 or 3 differentiation [Table 2].

Table 1: Demographic and clinical characteristics of the groups

	Mean±SD			*P*
	Group 1 (Kmax ≥52 dioptry, *n*=28)	Group 2 (Kmax ≥47.2 dioptry and <52 dioptry, *n*=30)	Group 3 (control, *n*=22)	
Gender (female/male)	14/14	15/15	11/11	>0.05
Age (years)	27.8±9.5	26.9±8.5	28±9.4	0.910
Kmax (dioptry)	59.5±8.7	49.5±1.8	44.8±1.2	<0.001
Thinnest (μm)	428.5±46.7	473.9±32.5	523.6±35.3	<0.001
TBUT (s)	6.9±3.1	7.3±3	11.8±1.6	0.012
Schirmer-I (mm)	13.3±8	16.6±9.2	21.7±9.1	0.012
OSDI score	41.1±25.7	35.3±23.6	19.1±10.2	0.006
CIC score	1.9±0.8	1.6±0.7	0.6±0.0.5	<0.001

TBUT: Tear breakup time, OSDI: Ocular surface disease index, CIC: Conjunctival impression cytology, SD: Standard deviation

Table 2: Conjunctival impression cytology grades of the groups

	Group 1 (Kmax ≥52 dioptry, *n*=28)	Group 2 (Kmax ≥47.2 dioptry and <52 dioptry, *n*=30)	Group 3 (control, *n*=22)
Grade 0 (*n*)	0	2	10
Grade 1 (*n*)	10	11	12
Grade 2 (*n*)	10	14	0
Grade 3 (*n*)	8	3	0

CIC: Conjunctival impression cytology

When Group 1 and Group 2 compared, the mean Kmax value was significantly higher in Group 1 than in Group 2 [$P < 0.001$, Table 1] and the mean thinnest value was significantly less in Group 1 than in Group 2 [$P < 0.001$, Table 1]. The mean Schirmer-I test value was lower in Group 1 and the mean OSDI score was higher in Group 1 than in Group 2 but the difference was insignificant ($P > 0.05$). Furthermore, the mean CIC score was higher in Group 1 than in Group 2 but the difference was insignificant [$P > 0.05$, Table 1].

Discussion

The traditional belief is that KC is a noninflammatory disease due to the absence of classical signs of inflammation.[1] However, in recent studies, it has been shown that levels of several inflammation mediators, interleukin (IL), cytokines, and proteolytic enzymes are increased in the tears and ocular surface of KC patients.[6,13-18] Many cytokines, primarily IL-1 alpha (IL-1α) and IL-1 beta (IL-1 β) are secreted by corneal epithelium due to corneal trauma and inflammation.[13,14] Curiously, it is found that IL-1α and IL-1 β are upregulated in KC corneas.[15,16] Another important pathogenic factor in systemic and corneal inflammation is tumor necrosis factor-alpha (TNF-α). Furthermore, elevated levels of TNF-α are found in the tear and corneal samples of KC patients. In addition, it is demonstrated that interleukin-17 (IL-17) is associated with corneal inflammation and it is shown that levels of IL-17 are increased in the tears of KC patients.[17]

The other ocular surface disorder that is associated with multifactor is DED.[19] Recent researches have confirmed that inflammation plays a crucial role in the development of DED.[19,20] The levels of IL-1ß, IL-6, IL-8, IL-10, TNF-α, and interferon-gamma are found to be elevated in the tears of DED patients when compared with healthy subjects.[21,22] The inflammation in the ocular surface may lead to conjunctival squamous metaplasia and tear film instability.[23] Indeed, many authors published that the levels of inflammatory cytokines in the tears of DED patients decrease with anti-inflammatory medications.[23,24] Furthermore, it is shown that goblet cell density increased after topical anti-inflammatory therapy.[25]

In the present study, we investigated whether there was an alteration in the tear film and morphological changes in the conjunctival epithelial cells in patients with mild or severe KC and its association with DED. We found that the tear function tests including TBUT and Schirmer-I test values were significantly lower in Group 1 and Group 2 when compared with control subjects. Indeed, the results were lower in Group 1 (severe KC) than in Group 2 (mild KC) but the differences were statistically insignificant.

The relationship between DED and KC was evaluated in several studies but the outcomes still remain controversial. In a pilot study, it was demonstrated TBUT values were insignificantly lower in 73% of KC patients and Schirmer-I test values were lower but the difference was slightly significant.[8] In another study, corneal sensitivity was evaluated by corneal esthesiometry, the tear film was investigated by Schirmer-I test and TBUT in KC patients.[26] In this study, researchers showed that corneal sensitivity and Schirmer-I test significantly lower in KC patients but there was no significant difference in TBUT between KC patients and healthy subjects. They found that there was no relation/correlation between corneal sensitivity and the tear film tests with KC severity. In a previous study, it has been reported that TBUT scores were significantly lower in 70% of the patients with moderate and severe KC.[7] Besides, there were no significant differences in Schirmer-I test values between KC patients and healthy subjects.[13] In addition, the authors noted that their hypothesis for the lower TBUT score was the corneal irregularity/topographic steepening of the cornea.[7] However, Zemova *et al*. investigated whether there was an interaction between corneal topographic/tomographic parameters and DED in KC and concluded that there was no relation between DED and topographic/tomographic alterations in KC.[27]

The other possible explanations of lower TBUT scores were the reduction of goblet cells and the variation of the quality/quantity of mucin secretion.[7] The stabilization of the tear film is ensured primarily by the mucin secreted by goblet cells.[28] The reduction of goblet cells and alterations of conjunctival epithelial cells disturb the balance of the tear film.[29] For this reason, we performed a CIC analysis to assess the changes in the ocular surface. CIC is a useful technique which enables clinicians to evaluate the epithelial cell morphology, assess nuclear and cytoplasmic characteristics, and quantify the goblet cell density in the conjunctiva.[9,10] We found that there was more squamous metaplasia and goblet cell loss in the bulbar conjunctiva in Group 1 and Group 2 when compared with control subjects. Indeed, the CIC score was higher in Group 1 (severe KC) than in Group 2 (mild KC) but the difference was statistically insignificant. The statistical power to detect differences between patients with severe and mild KC was limited due to the small number size of patients. However, the results may be significant in the studies including a larger number of subjects.

Very few studies have investigated the relationship between the severity of KC with DED and performed CIC to correlate these diseases.[7,8] In one study, it was reported that the cytologic changes were more conspicuous in patients with severe KC.[7] In addition, another study showed goblet cell loss by CIC in a small number size of KC patients when compared with healthy subjects.[8]

The last test in our study for evaluating dry eye symptoms in KC patients is the OSDI questionnaire. OSDI is a reliable questionnaire for diagnosing DED and grading the severity of dry eye signs and symptoms.[30] The mean OSDI score was significantly higher in patients with severe KC (Group 1) in our study, confirming the results of previous studies. In recent studies, OSDI scores were higher in KC patients when compared with healthy subjects.[8,26]

The small number size of patients and single-center study design are the limitations of this study but our results may indicate that there is a relation between the severity of KC and dry eye symptoms. Inflammatory processes caused by DED may be exacerbating inflammation on the ocular surface of KC patients and this condition may probably affect the severity of KC.

Conclusion

We have discussed the association between the severity of KC and DED in the present study. Despite research opportunities and advanced technology, today, there are still many questions that need to be answered. The core mechanism of KC is not well known yet. Nevertheless, after consideration of the results of our study and the outcomes of previous studies mentioned above KC patients have inflammatory ingredients in the tear film and the ocular surface. Probably, KC origins from inflammatory processes. For this reason, it appears to be inappropriate to define KC as a noninflammatory disease. Moreover, the inflammation in DED may affect the corneal microenvironment and leads to severe KC. Further studies are needed to elucidate the development mechanisms of KC and evaluating the ocular surface; the tear film and the conjunctiva cells may throw light on the pathogenesis of KC.

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Conflicts of interest
There are no conflicts of interest.

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