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Original Article

Meibomian Gland Alterations in Keratoconus Patients After Corneal Cross-Linking

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Abstract

Objectives: The objective of the study was to evaluate the changes in the meibomian glands (MGs) and ocular surface parameters after corneal cross-linking (CXL) in keratoconus patients.

Methods: Forty-eight eyes of 48 keratoconus patients that underwent epi-off CXL were included in this prospective study. Upper and lower lid MGs were assessed with non-contact meibography at preoperatively, 1st, 3rd, 6th, and 12th month after CXL. Uncorrected distance visual acuity (UCVA), corrected distance visual acuity (BCVA), spherical equivalent (SE), and corneal tomography findings (K1, K2, Kmean, and Kmax) were recorded at each visit. Ocular surface staining score (Oxford grade), ocular surface disease index (OSDI) questionnaire, and non-invasive tear break-up time (NI-TBUT) were evaluated at preoperatively and 12 months after CXL.

Results: K1, K2, Kmean, and Kmax were decreased at post-operative 12th month compared to baseline ($p=0.004$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). UCVA, BCVA, and SE did not change between preoperatively and post-operative 12 months ($p=0.142$, $p=0.306$, and $p=1.000$, respectively). NI-TBUT showed similarity between pre-operative and 12 months values ($p=0.180$), while OSDI scores significantly decreased ($p<0.001$). MG loss in the upper and lower lids did not show significant difference compared to pre-operative values at any of the follow-up visits ($p=0.121$ and $p=0.117$, respectively).

Conclusion: CXL treatment did not significantly affect the NI-TBUT and MGs morphology, while improving ocular symptoms.

Keywords: Corneal cross-linking, Keratoconus, Meibography

Introduction

Corneal cross-linking (CXL) is a widely used, minimally invasive procedure that stabilizes the cornea in keratoconus disease by strengthening the corneal collagen matrix through photochemical reactions with ultraviolet-A (UVA) and riboflavin (1). Although it is an effective and gold standard treatment for preventing progression of keratoconus, epithelial removal and UV exposure lead to corneal nerve damage in the post-operative period, resulting in decreased corneal sensitivity (2-4). Since the effect of corneal nerves on ocular surface homeostasis is crucial, several studies have been performed to investigate ocular surface parameters after CXL (5-8). Some of these studies have found no change in ocular surface parameters following CXL, (5,6) while others indicated improvement (7,8). Voronin et al. observed a significant decrease in tear break-up time 1 month after CXL; however, by the 6th month, the tear

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break-up time was comparable to pre-operative values (9). The findings of the aforementioned studies indicate that CXL does not have a detrimental impact on ocular surface parameters.

Corneal structural alterations associated with CXL procedure have been well documented in previous reports (2,3). Another structure potentially affected by CXL is the meibomian glands (MGs), which are modified sebaceous glands located in the upper and lower eyelids. Each MG is comprised of a central duct, which opens at the margin of the lid. The ductules divert laterally from the central duct and each terminate in the acini which are connected to capillaries, nerves, and fibroblasts. The innervation of the MGs is derived from different sources, including parasympathetic, sympathetic, and sensory fibers originating from the trigeminal ganglion (3). The close proximity of these glands to vessels and nerves in surrounding tissues may suggest a possible influence of CXL on their function. However, there are a limited number of studies in the literature on this topic (6,10). Understanding these interactions is crucial for optimizing treatment outcomes and managing ocular surface health in CXL patients. The aim of this study was the long-term evaluation of changes in MGs morphology and ocular surface parameters after CXL.

Methods

This prospective study was conducted at the Department of Ophthalmology, Marmara University School of Medicine, İstanbul, Türkiye. The study was approved by the Institutional Review Board and adhered to the tenets of the Declaration of Helsinki and Good Clinical Practice (Protocol No: 07.2024.905). Before enrollment, informed written consent was obtained from all study subjects.

This study included 48 eyes from 48 patients over the age of 18, all diagnosed with progressive keratoconus and recommended for CXL. Exclusion criteria were corneal thickness below 400 µm, pregnancy, breastfeeding, use of topical or systemic medications, presence of eye diseases other than keratoconus (including dry eye disease), systemic diseases, active atopy or allergies, contact lens wear, and a history of ocular surgery. A comprehensive ophthalmic examination was performed for all patients, which included uncorrected distance visual acuity, manifest refraction, corrected distance visual acuity, slit-lamp biomicroscopy, and fundus examination. Keratoconus stages were determined by clinical evaluation and corneal tomography (Pentacam, OCULUS, Wetzlar, Germany) according to the Amsler-Krumeich classification (11). All patients underwent epithelium-off CXL for 10 min with 9 mW/cm² UVA irradiation. Postoperatively, all patients were treated with topical moxifloxacin 0.5% (Vigamox; Alcon Laboratories, Inc.) 4 times daily for 1 week and preservative-free artificial eye drops. After epithelial closure, topical dexamethasone (Dexa-sine Se 0.4 mL/1.3 mg, Kaysersberg Pharmaceuticals France) was initiated 4 times daily for 1 week, followed by loteprednol (Lotemax; Bausch & Lomb, USA) 4 times daily for 3 weeks.

Ocular surface parameters including the ocular surface disease ındex (OSDI), questionnaire, non-invasive tear break-up time (NI-TBUT), and ocular surface staining were assessed preoperatively and 12 months after CXL. The Turkish-validated version of the OSDI scoring was used (12). The fourth and fifth questions in the first section of the questionnaire, regarding blurry vision and reduced visual symptoms, were excluded as these symptoms may already be present in patients with keratoconus (6). The total OSDI score was calculated according to the formula: OSDI = ((sum of scores for all questions answered) × 100)/((total number of questions answered) × 4). NI-TBUT was assessed using a Sirius Scheimpflug camera (CSO, Florence, Italy) and the device automatically provided the average NI-TBUT value. Ocular surface staining was evaluated using sterile fluorescein strips. Staging was performed according to the Oxford scheme (13).

The MGs were evaluated using the camera recording system of the Sirius topography device (C.S.O., Florence, Italy). The patient's head and jaw position were adjusted, and the upper eyelid was expanded with an applicator to photograph the area where the MGs were clear. The same procedure was performed on the lower eyelid. The Phoenix-Meibography Imaging Module in the instrument software recorded the images for analysis. The tarsal region borders were marked at four points, and the edges of intact MGs within this area were manually outlined. The software automatically determined the percentage and degree of the loss area. MGs were assessed before CXL and 1, 3, 6, and 12 months postoperatively. Manifest spherical equivalent (SE), uncorrected distance visual acuity (UCVA), corrected distance visual acuity (BCVA), and tomography findings, including thinnest corneal thickness (TCT), K1, K2, Kmean, and Kmax, were also recorded at each visit.

Statistical Analysis

The SPSS statistical software (version 21.0, IBM Corp., Armonk, NY, USA) was used for data analysis. Descriptive statistics are presented as mean±standard deviation or median and 95% confidence intervals. Categorical variables were described as frequency and percentage. Normality of data distribution was determined through the Kolmogorov–Smirnov test and histogram graphs. For analysis of multiple dependent variables over time, repeated measures analysis of variance (ANOVA) or the Friedman test was employed. Post hoc analyses for repeated measures involved repeated

measures ANOVA with Bonferroni-Dunn correction or the Friedman test, Pairwise Comparison, with Bonferroni correction. Pearson Chi-square test was used to compare categorical data. For correlation analysis, Spearman correlation test was used. P-value below 0.05 was considered statistically significant.

Results

The study included 17 females (35.4%) and 31 males (64.6%), with a mean age of 23.10±3.02 years. Twenty-six eyes (54.2%) were stage 1, 18 eyes (37.5%) were stage 2, and 4 eyes (8.3%) were stage 3.

There were no significant differences in UCVA, BCVA, and SE between the pre- (0.40±0.26 logMAR, 0.27±0.17 logMAR, and −4.21±1.86 D, respectively) and post-operative 12 months (0.35±0.27 logMAR, 0.23±0.16 logMAR, and −4.10±2.02 D) values (p=0.142, p=0.306, and p=1.000, respectively). K1, K2, Kmean, and Kmax were decreased at post-operative 12th month compared to baseline (Table 1). The mean changes in K1, K2, Kmean, and Kmax were as follows: −0.38±0.64 D, −0.51±0.64 D, −0.43±0.49 D, and −0.95±1.04 D at 12th month, respectively.

Oxford grade was 0 at both pre-operative and 12-month post-operative follow-up in all eyes. There was no difference between the pre-operative (11.56±4.70 s) and post-operative NI-TBUT values at 12 months (12.80±4.18 s) (p=0.180). OSDI score decreased at post-operative 12 months (4.43±5.68) compared to baseline (13.71±5.06) (p<0.001).

Upper lid, meibography drop-out score was Grade 0 in 26 eyes, Grade 1 in 18 eyes, and Grade 2 in 4 eyes preoperatively. The drop-out score of the lower lid meibography was Grade 1 in only one eye, while it was Grade 0 in the other eyes at the baseline. The percentage of MG loss in the upper and lower lids did not show significant change compared to pre-operative values at any of the follow-up visits (Table 2 and Fig. 1). There is no difference in the upper and lower lid MG loss between keratoconus stages (p=0.278, p=0.395, respectively). No significant correlation was observed between MG loss and the stage of keratoconus (upper eyelid; r=−0.069 p=0.643, p=0.898, lower eyelid; r=−0.250 p=0.087).

Table 1. Comparison of pre- and post-operative keratometry values

	Pre-operative[a]	**1st month[b]**	**3rd month[c]**	**6th month[d]**	**12th month[e]**	**P1[†]**	**P2[‡]**
K1 (D)	45.48±2.53	45.35±2.72	44.88±2.63	45.00±2.50	45.05±2.51	<0.001	a vs. b=1.000
							a vs. c<0.001
							a vs. d=0.004
							a vs. e=0.004
K2 (D)	49.15±2.94	49.20±3.12	48.61±3.18	48.64±2.90	48.55±2.86	<0.001	a vs. b=1.000
							a vs. c=0.001
							a vs. d=0.005
							a vs. e<0.001
Kmean (D)	47.22±2.54	47.19±2.77	46.65±2.71	46.75±2.52	46.72±2.43	<0.001	a vs. b =1.000
							a vs. c <0.001
							a vs. d=0.001
							a vs. e <0.001
Kmax (D)	54.34±4.28	54.88±4.90	53.80±4.64	53.67±4.50	53.39±4.22	<0.001	a vs. b=0.299
							a vs. c=0.110
							a vs. d=0.013
							a vs. e<0.001
TCT (µm)	463.41± 31.54	436.75 ± 39.35	443.88 ± 37.36	451.05 ± 35.99	461.75 ± 34.94	<0.001	a vs. b<0.001
							a vs. c<0.001
							a vs. d=0.001
							a vs. e=1.000

TCT: Thinnest corneal thickness; [†]Repeated measures ANOVA test; [‡]Repeated measures ANOVA test; Pairwise comparison-Bonferroni correction; Values are presented as mean±standard deviation, P-values in bold are statistically significant, P2-values are adjusted P-values after Bonferroni correction at a significance level of 0.05. ANOVA: Analysis of variance.

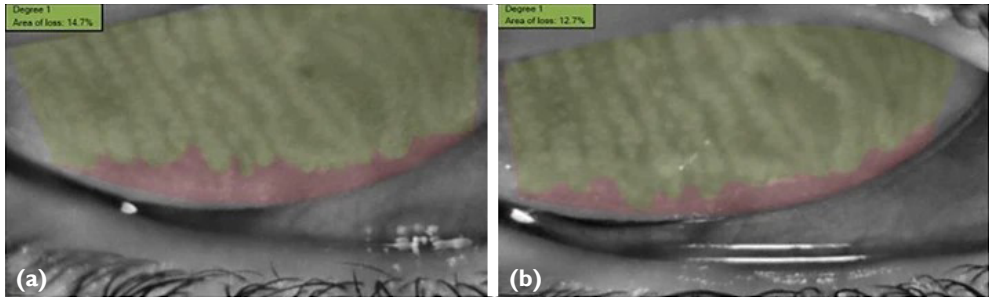

Figure 1. Representative images of meibomian glands in the upper lids of a patient obtained by meibography **(a)** before cross-linking and **(b)** 12 months after cross-linking.

Table 2. The meibomian gland morphological alterations at follow-ups

	Upper lid MG loss percentage	Lower lid MG loss percentage
Pre-operative	10.51±8.05	3.19±1.69
1st month	10.45±7.85	3.12±1.64
3rd month	10.80±8.10	3.08±1.56
6th month	10.84±8.17	3.14±1.65
12th month	10.53±7.96	3.11±1.58
p†	0.121	0.117

MG: Meibography; †Friedman test, Values are presented as mean±standard deviation P-values in bold are statistically significant.

Discussion

In this study, we evaluated MG loss in keratoconus patients and assessed the effect of CXL in the long term. Our results indicated that CXL did not have a detrimental effect on ocular surface parameters. Moreover, there was an improvement in subjective symptoms. Since no changes in MG morphology were observed at any follow-up time, it can be concluded that CXL does not cause long-term damage to the MGs.

The effects of direct application of the CXL procedure on tarsus have been evaluated in ex vivo studies (14-16). Smith et al. found no structural changes in the MGs when CXL was applied to the ovine tarsi at different irradiation levels ranging from 9 to 22.5 J/cm² (15). In addition, the study conducted by Smith et al. reported better protection of MGs after irradiation of the surrounding tissues which showed a more compact structure with CXL (15). However, at higher irradiance levels (250 mW/cm^2, equivalent to a fluence of 45 J/cm^2), damage to both the surrounding tissues and the MGs were observed.(15) In an ex vivo study conducted by Ugradar et al. on human tarsus, no structural differences were observed in the MGs after CXL at a fluence of 6.48 J/cm^2 compared to the control group (14). It is important to note that in these ex vivo studies, the acute damage caused by UVA radiation has been investigated primarily. Therefore, in vivo studies with long-term evaluations are more appropriate for assessing the chronic UVA damage that may develop in the MGs.

The previous studies have shown that MG dysfunction is more common in keratoconus patients than in the healthy population, with higher meibography, dropout, and distortion scores (17-20). In contrast to previous studies, MG loss was not associated with the severity of keratoconus in our study (17,19,20).

Akgöz et al. evaluated both ocular surface parameters and MG morphology in keratoconus patients compared to healthy subjects and investigated the effect of CXL on these parameters for a period of 6 months (6). In this study, it was observed that keratoconus patients had lower TBUT, higher OSDI, and higher meibography scores than the healthy population (6). However, CXL did not lead to deterioration in any of these values (6). Similar to our study, Balıkçı and Ulutaş evaluated the effects of epi-off CXL (9 mW/5.4 J/cm^2) on ocular surface parameters and MGs morphology (10). They observed that NI-TBUT decreased at 1 month post-CXL but reached to preoperative values at 6 months (10). In addition, no change was observed in the OSDI score (10). In their study, the authors have focused only on lower lid MG morphology, suggesting that MG dysfunction may progress more rapidly in the lower lid than in the upper lid (10). In our study, we evaluated the effects of CXL on MG morphology over a 12-month period, assessing both upper and lower eyelid morphology.

We found that, there was a decrease in the OSDI score, although the NI-TBUT remained stable after surgery. The improvement in subjective symptoms may be attributed to the regularization of the cornea and the formation of a healthier epithelium following CXL (21).

Our results showed that MG loss was not associated with the severity of keratoconus, which may be due to the fact that the majority of our patients were in Stages 1 and 2. Increasing the sample size might include more advanced stages of keratoconus. One of the major limitations of our study is the lack of a healthy control group. In addition, the evaluation of MGs was morphological only, without assessing meibum function and quality. Furthermore, corneal sensitivity and corneal

nerve regeneration could have been assessed for their effect on MG components and ocular surface parameters. Despite its limitations, this study provides valuable insight into the safety of CXL treatment on MG over a 12-month period.

Conclusion

Based on this study, it is evident that CXL does not have a significant long-term impact on the MGs. These findings contribute to a better understanding of the potential effects of CXL on MG function and underscore the importance of further research in optimizing treatment outcomes.

Disclosures

Ethics Committee Approval: This prospective study was conducted at the Department of Ophthalmology, Marmara University School of Medicine, İstanbul, Türkiye. The study was approved by the Institutional Review Board and adhered to the tenets of the Declaration of Helsinki and Good Clinical Practice (Protocol No: 07.2024.905).

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