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Research Article

Investigation of Epithelial Thickness Profile Using Optical Coherence Tomography (OCT) After Corneal Collagen Cross-linking for Keratoconus

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Abstract

Purpose: To evaluate the epithelial thickness profile changes using optical coherence tomography (OCT) after corneal collagen cross-linking (CXL) for keratoconus.

Methods: Thirty eyes of 30 keratoconus patients (13 males and 17 females) with the mean age of 21.6 ± 4.1, who were candidates for CXL were examined. Before and after CXL, patients underwent corneal epithelial thickness measurement with anterior segment optical coherence tomography (AS-OCT) and the results were compared.

Results: The best-corrected visual acuity (BCVA) was changed from 0.33 ± 0.26 logarithm of the minimum angle resolution (logMAR) to 0.17 ± 0.29 logMAR at 6 months after surgery ($p < 0.001$). The corneal epithelial thickness in the superior was significantly reduced after surgery (55.2 ± 3.8 µm and 51.73 ± 3.73 µm, respectively, $p < 0.001$). The epithelial thickness in the inferotemporal was also significantly reduced after surgery (55.05 ± 4.6 µm and 51.57 ± 3.35 µm, respectively, $p < 0.001$). The ratio of the superior and inferior epithelial thickness was significantly decreased from 1.02 ± 0.07 to 0.96 ± 0.03 at 6 months after surgery ($p < 0.001$).

Conclusions: This study demonstrated that CXL improved the vision of keratoconus patients. The corneal epithelial thickness decreased in all sectors that were statistically significant in the superior and inferotemporal areas. It seems that the epithelial thickness evaluation is important in both treated and untreated patients and gives beneficial information about the corneal biomechanics and its remodeling.

Keywords: Keratoconus; Collagen Cross-Linking; Corneal Epithelial Thickness; Visual Acuity; OCT

Background

Keratoconus is a progressive and noninflammatory bilateral corneal ectasia. It manifests with an abnormal thinning of the corneal stroma, conical protrusion, and a gradual decrease in visual acuity. Its worldwide prevalence varies widely from 0.3 to 2300 per 100,000 in the general population (approximately 1 per 2,000) [1-3]. Its clinical signs have been well described but the early forms of the disease may go undetected at first unless the anterior corneal topography is done earlier [4]. The significant risk factors for keratoconus development include ocular allergy, atopic dermatitis, some connective tissue disorders, diabetes mellitus (DM), and Down syndrome. Some reports have shown the greater incidence of keratoconus in Asians [5,6]. Early diagnosis of keratoconus (subclinical and asymptomatic forms) is so important especially before doing refractive surgery and prevents progression of the disease [7]. Any patient with significant irregular astigmatism increasing over time should be evaluated for keratoconus [8]. Corneal topography and tomography (Scheimpflug imaging or anterior segment optical coherence tomography, OCT) evaluate the anterior and posterior corneal surfaces and generate corneal thickness, and elevation maps, epithelial imaging, and analysis of the anterior segment of the eye [9-11]. Corneal CXL with riboflavin and UVA has great importance to slow or even to inhibit the progression of keratoconus. It could be done with (epi-off) or without (epi-on) removal of the epithelium [12]. Nowadays, measuring profiles of corneal epithelium thickness is becoming more important in the diagnosis of

subclinical disease, monitoring of keratoconus progression, and in corneal therapeutic refractive surgery [13,14]. There are several studies about the epithelial thickness changes after CXL. Epithelial and stromal remodeling after CXL may facilitate the evaluation of the corneal curvature and thickness after CXL [13,15,16]. Anterior segment optical coherence tomography (AS-OCT) is commonly used for the analysis of the corneal epithelial thickness changes in keratoconus, postoperative corneal ectasia, and in normal eyes [13,17]. The epithelial thickness profile in keratoconus has a greater range of deviation than in a normal healthy eye. Therefore, the comparison of the corneal thickness profile before and after CXL may give some information about the epithelial remodeling in keratoconic eyes [13,18]. So in this study, we decided to analyze the corneal topographic changes and epithelial remodeling after epi-off CXL in keratoconus patients in the Azarbaijan area.

Materials and Methods

In this retrospective study, we enrolled 30 eyes of 30 patients diagnosed with keratoconus at the Nikookary Eye Hospital, Tabriz University of Medical Sciences, Iran from December 2019 to July 2021. We selected the Amsler-Krumeich (AK) system [19] for the keratoconus classification (Table 1). Based on refractive error, central keratometry, presence or absence of scarring, and central corneal thickness, the AK system classifies keratoconus into four stages.

Table 1: Amsler–Krumeich classification system.

Stage	Findings
1	Eccentric steepening, Myopia/astigmatism < 5.00 D, Mean K < 48.0 D
2	Myopia/astigmatism > 5.00 D but < 8.00 D, Mean K < 53.00 D, Absence of scarring, Corneal thickness > 400 µm
3	Myopia, induced astigmatism > 8.00 D but < 10.00 D, Mean K > 53.00 D, Absence of scarring, Minimal apical corneal thickness <400 µm but > 300 µm
4	Refraction not measurable, Mean K > 55.00 D, Central corneal scarring, Minimal apical corneal thickness < 300 µm

All these patients were candidates for epi-off CXL due to evidence of progressive keratoconus such as increases in the maximum K reading (K_{max}) according to corneal tomography imaging using rotating Scheimpflug corneal topography (Pentacam HR, Oculus GmbH, Wetzlar, Germany) or increases in astigmatism and refractive error. Patients with current infection, history of ocular herpesviruses infections, autoimmune disorders, history of poor epithelial wound healing, corneal scarring, a corneal thickness of fewer than 400 microns after epithelial removal, and also pregnant patients were excluded. The uncorrected and best-corrected visual acuity, slit-lamp examination findings, refractive and keratometric findings, and corneal thickness data by AS-OCT (Optovue Inc., Fremont, USA) were collected before and after surgery. Across a diameter of 6 mm, centered over the pupil image with AS-OCT, we analyzed the information of central, superior, inferior, inferotemporal, and inferonasal sectors with all inferior sectors before and after surgery (Figure 1).

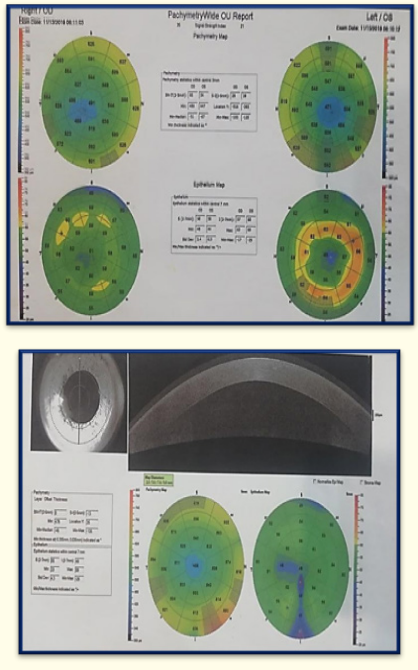

Figure 1: Corneal epithelial thickness mapping obtained by spectral-domain OCT.

The study was approved by the Institutional Ethics Committee of Tabriz University of Medical Sciences (approval number: IR.TBZMED.REC.1398.871) and was done according to the Ethical Principles of the Declaration of Helsinki. We informed all the patients or their legal guardians about the study aims, risks and potential benefits, confidentiality of their information, and voluntary nature of their participation in the format of written informed consent.

Surgical technique

All surgical procedures were done by one surgeon according to Dresden protocol. Under sterile conditions and topical anesthesia with 0.5% tetracaine hydrochloride, the corneal epithelium was

mechanically removed with a 9.0 mm diameter with a crescent knife. Then, riboflavin drops (0.1% riboflavin-5-phosphate and 20% dextran T-500) were instilled into the corneal surface every minute for 30 minutes. Ultraviolet A irradiation was performed 5.4 J/cm^2 (3 mW/cm^2) for 30 minutes at a working distance of 5 cm while continuing instilling riboflavin drops every 5minutes. Finally, a balanced salt solution was applied and a bandage contact lens was placed until complete re-epithelialization was achieved. All patients were prescribed topical 0.1% chloramphenicol four times a day for 5 days and 0.1% corticosteroid betamethasone four times a day for 2 weeks and preservative-free artificial tears four times a day for one month. Postoperative examinations were scheduled a day after surgery, 1 week, and at 1, 3, and 12 months postoperatively.

Statistical analysis

Analyses were performed using the SPSS software (ver.26.0 for Windows; SPSS, Inc., Chicago, IL, USA). The normality of numeric data was checked by the Shapiro–Wilk test. For the comparison of epithelial thickness datasets before and after surgery, the paired t-test or Wilcoxon signed-rank test was used. For the non-normal distributed data, the Wilcoxon rank-sum test was employed. The p-value <0.05 was considered to indicate statistical significance.

Results

In this retrospective study, 30 eyes of 30 keratoconus patients (13 males and 17 females) with the mean age of 21.6 ± 4.1 (range, 17-48 years) were enrolled. According to the AK system, the frequency of affected eyes in stages 1 to 4 was 12 (40%), 6 (20%), 7 (23%), and 5 (17%), respectively. Preoperative and postoperative refractive and topographic data are summarized in Table 2. UCVA improved significantly 6 months after CXL from 1.23 ± 0.29 logarithm of the minimum angle resolution (logMAR) to 0.9 ± 0.28 logMAR (P < 0.001). Also, BCVA changed significantly from 0.33 ± 0.26 logMAR to 0.17 ± 0.29 logMAR during the 6 months follow-up (P < 0.001).

We evaluated and analyzed the information of epithelial thickness in nine corneal sectors measured by AS-OCT. Table 3 demonstrates the preoperative and postoperative mean epithelial thickness data during 6 months' follow-up.

Table 2: Preoperative and postoperative refractive and topographic data.

	Pre-op (Mean ± SD)	3-month Post-op (Mean ± SD)	6-month Post-op (Mean ± SD)	P-value
UCVA (logMAR)	1.23 ± 0.29	098 ± 0.3	0.9 ± 0.28	<0.001
BCVA (logMAR)	0.33 ± 0.26	0.24 ± 0.3	0.17 ± 0.29	<0.001
Refractive sphere (D)	-3.05 ± 1.63	-2.56 ± 1.5	-2.39 ± 1.42	<0.001
Refractive cylinder (D)	4.43 ± 1.68	3.9 ± 1.6	3.5 ± 1.31	<0.001
SE (D)	-5.26 ± 1.87	-4.48 ± 1.73	-4.17± 1.7	<0.001
K_{min} (D)	49.87 ± 4.12	49.34 ± 4.23	49.33 ± 4.19	0.3
K_{max}(D)	50.76 ± 5.3	50.55 ± 5.36	50.31 ± 5.342	0.43
Pre-op: preoperative; Post-op: postoperative; UCVA: uncorrected visual acuity; BCVA: best corrected visual acuity; SE: spherical equivalent; K_{min}: minimum simulated keratometry; K_{max}: maximum simulated keratometry				

Table 3: Preoperative and postoperative mean corneal epithelial thickness data.

	Pre-op(Mean ± SD)	3-month Post-op (Mean ± SD)	6-month Post-op (Mean ± SD)	P-value
Superior	55.2 ± 3.8	52 ± 3.58	51.73 ± 3.73	<0.001
Superotemporal	53.87 ± 5.54	53.25 ± 4.95	53.77 ± 3.98	0.43
Superonasal	55.32 ± 3.98	54.94 ± 3.53	55.05 ± 4.87	0.67
Inferior	51.43 ± 4.9	50.65 ± 4.87	51.01 ± 4.65	0.51
Inferotemporal	55.05 ± 4.6	51.6 ± 3.4	51.57 ± 3.35	<0.001
Inferonasal	49.35 ± 4.54	48.12 ± 4.86	49.1 ± 4.02	0.5
Center	49.9 ± 4.62	49.2 ± 4.17	50.05 ± 0.13	0.91

Temporal	54.3 ± 5.05	53.75 ± 3.87	54.01 ± 4.34	0.45
Nasal	54.05 ± 3.9	53.55 ± 3.86	52.92 ± 3.81	0.24
The mean of inferior sectors*	51.81 ± 3.34	50.85 ± 3.75	50.51 ± 3.64	0.137
Superior/Inferior	1.02 ± 0.07	0.99 ± 0.03	0.96 ± 0.03	<0.001
Pre-op: preoperative; Post-op: postoperative *Inferior, Inferotemporal and Inferonasal sectors				

As figure 2 shows, the superior corneal epithelial thickness was significantly reduced 6 months after surgery (55.2 ± 3.8 and 51.73 ± 3.73, respectively, $p < 0.001$). The inferotemporal corneal epithelial thickness was also significantly reduced 6 months after surgery (55.05 ± 4.6 and 51.57 ± 3.35, respectively, $p < 0.001$). The ratio of the superior /inferior corneal thickness was significantly reduced 6 months after surgery (1.02 ± 0.07 and 0.96 ± 0.03, respectively, $p < 0.001$; Figure 3). In other corneal sectors, the corneal thickness changes were not statistically significant.

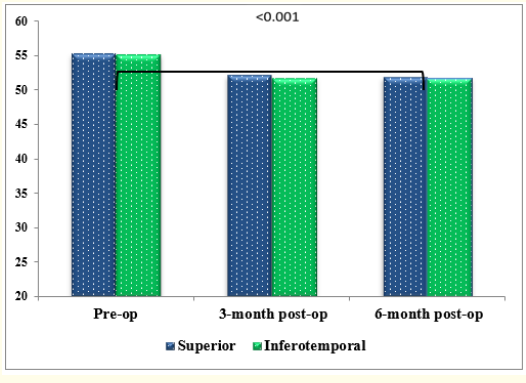

Figure 2: The trend of epithelial thickness changes in both superior and inferotemporal corneal sectors preoperatively (Pre-op) and 3 and 6 months postoperatively (post-op).

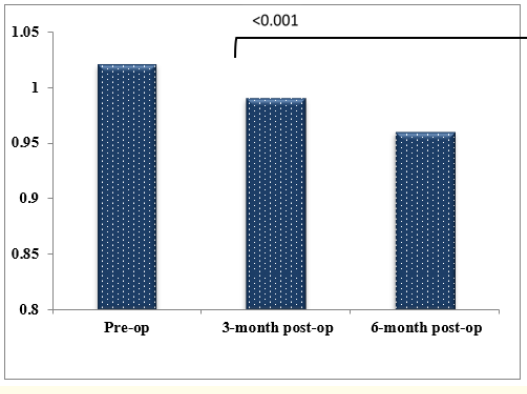

Figure 3: The ratio of the superior/inferior epithelial thickness preoperatively (Pre-op) and 3 and 6 months postoperatively (post-op).

Discussion

Keratoconus is a bilateral and degenerative disorder of the cornea that is characterized by progressive distortion of the anterior corneal surface and apical thinning of the stroma [20-22]. The CXL, introduced by Wollensak., *et al*. in 2003, ceases the progression of keratoconus. If the corneal thickness is at least 400 microns after de-epithelialization, CXL will be recommended. The pachymetry of fewer than 400 microns is usually found in moderate-to-advanced cases [23]. The CXL increases the corneal biomechanical stability and is commonly used for the management of progressive keratoconus [24]. AS-OCT demonstrates irregularity and alterations in regional epithelial and total corneal thickness profiles in both keratoconus and ectatic eyes [17]. Epithelial thickness maps can also give useful information for monitoring the progression of corneal ectasia after CXL [15].

In normal eyes, the corneal epithelium is slightly thinner in the superior area. In keratoconus patients, a doughnut pattern of epithelial thinning could be seen over the cone [25]. It seems that epithelial and total corneal pachymetry measurements increase before and after CXL surgery [26].

In this study, we analyzed the corneal epithelial thickness before and in 6 months follow-up evaluation in patients treated with accelerated CXL for progressive keratoconus. After treatment by CXL, significant improvement in UCVA and BCVA and a significant reduction in refractive sphere and cylinder occurred.

There are several studies about corneal epithelial thickness profile using AS-OCT in keratoconic eyes [11,13-15,27,28]. Chen., *et al*. [13] evaluated the epithelial thickness changes after combined topography-guided transepithelial photorefractive keratectomy and CXL in keratoconus. 6 months postoperatively, they found epithelial thickening of 5.5 ± 5.1 μm at the thinnest area by RTVue-100 AS-OCT. The epithelial thickness in other areas was not significantly different and the difference in epithelial thickness between the thinnest area and the rest of the paracentral areas decreased to 5.5 ± 4.3 μm. In other areas, the thickness remained unchanged.

In another study, Junjie., *et al*. [14] found the significant epithelial thickness decrease at 6 months after epi-off CXL in these sectors: inner nasal, inner superior-nasal, inner superior, inner superior-temporal, outer inferior, outer inferior-nasal, and outer temporal. The corneal stromal thickness changed but the preoperative and postoperative mean CCT values didn't change statistically significant. In our study, the thickness of the corneal epithelium was significantly reduced in the superior and inferotemporal sectors.

In a study by Reinstein., *et al*. [15], the epithelial thickness profile by an ultrasound scanning showed a slight decrease in the area of epithelial thinning and decreased peripheral thickening after CXL. The changes in minimum stromal and corneal thicknesses were not significant.

The one study by Rocah., *et al*. [16] AS-OCT significantly demonstrated thinner epithelial thickness 2.5 and 2 mm below and 1.5 mm above the corneal apex and 2.5 and 1 mm nasal and 2 mm temporal to the corneal apex after CXL. They observed a significant remodeling of the corneal epithelium after CXL in the eyes with keratoconus.

Haberman., *et al*. [27] demonstrated that at 12 months after CXL, the corneal epithelium was significantly thinned in inferior and nasal regions by 1.1 to 3.2 μm (outer and inner nasal, outer and inner inferonasal, outer inferior, outer inferotemporal, and outer temporal). The pattern of epithelial thickness changes would be related to more affected areas by CXL. They found a significant improvement in corneal surface regularity across the central 6.0 mm, by 6 months after treatment in patients with progressive keratoconus.

Zhang., *et al*. [28] found a decrease in epithelial thickness during the first week after CXL for keratoconus patients. The postoperative epithelial thickness, measured by RTVue-100 AS-OCT, was not significantly different up to 12 months. After treatment, the epithelial thickness decreased for a while, then stabilized at month 12. The minimum number of epithelial points changed from 41.68 ± 8.78 μm to 40.96 ± 10.27 μm while the maximum number of epithelial points' changes was from 65.57 ± 7.10 to 65.26 ± 6.22 at 12 months.

Unlike the mentioned studies, Kanellopoulos., *et al*. [29] showed that epithelium thickness distributions are similar to the healthy eyes, rather than the keratoconus. In some cases, the epithelium was normal while others showed local variations. They suggested epithelial hyperplasia as a hypothesis for biomechanically unstable corneas.

This study showed that cross-linking improves patients' vision. UCVA and BCVA improved significantly and the spherical equivalent decreased at 6 months after CXL. During this follow-up period, the corneal epithelial thickness significantly decreased in the superior and inferior areas in the temporal quadrant. In other areas of the cornea, no significant changes were observed in the thickness of the corneal epithelium before and after cross-linking. However, all of them had a decrease in epithelial thickness at 3 and 6 months after treatment.

The corneal epithelium is thicker in ectatic areas as a hypertrophic modification of abrupt changes in stromal thickness. It seems that the significant epithelial thickness changes after CXL is seen in treated areas and the superior area remains less affected. The inferotemporal sector changes are consistent with these findings. However, the superior corneal epithelial thickness in our study had a different trend line [27,30,31]. Some studies have demonstrated a downward trend in epithelial thickness after CXL [13-16,27,28] while others showed a thicker corneal epithelium or unchanged thickness in some sectors after treatment [29]. These differences may have some reasons: first, the distribution of irregularity in the corneal surfaces may be different between patients. Second, the corneal cross-linking procedures may differ by study. Third, as we mentioned, combined transepithelial photorefractive keratectomy and CXL in the treatment of keratoconus [13] could create a different postsurgical epithelial remodeling pattern.

In this study, we showed a different pattern of epithelial remodeling after CXL in keratoconic eyes. The evaluation of epithelial thickness profile after CXL will help us understand more about the epithelial remodeling after treatment in keratoconus and will improve the long-term efficacy and effectiveness of treatments. Further studies with a longer follow-up and larger sample size will make it more possible in the future.

Conclusion

In this study, CXL vastly improved the visual acuity and decreased the epithelial thickness in all corneal sectors that were statically significant in the superior and inferotemporal areas. It seems that the epithelial thickness evaluation is important in both

treated and untreated keratoconic eyes and gives beneficial information about the corneal biomechanics and its remodeling after surgical interventions.

Conflict of Interest

None to declare.

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