Demarcation Line Evaluation of Iontophoresis-Assisted Transepithelial Corneal Collagen Cross-linking for Keratoconus

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ABSTRACT

PURPOSE: To evaluate the visualization and depth of the demarcation line with anterior segment optical coherence tomography (AS-OCT) after iontophoresis-assisted transepithelial corneal collagen cross-linking (CXL).

METHODS: This prospective, consecutive, single center, non-randomized clinical study involved 15 eyes of 12 patients with keratoconus who underwent an AS-OCT scan (Spectralis; Heidelberg Engineering, Inc., Carlsbad, CA) to search for a demarcation line and its depth at 1 month after iontophoresis-assisted transepithelial CXL. AS-OCT scan measurements were performed by two independent examiners.

RESULTS: No intraoperative or postoperative complications were observed. Kappa coefficient estimation for operator agreement in demarcation line visualization (whether it was visualized) was 70.6%. The corneal stromal demarcation line was identified in 9 eyes (60%) by both examiners. Mean depth of the corneal stromal demarcation line was 246.67 ± 50.72 μm (range: 183 to 339 μm) for the first examiner and 241.89 ± 62.52 μm (range: 163 to 358 μm) for the second examiner. There were no statistically significant differences for the measurements of the paired comparisons between the two examiners ($P = .61$). The Pearson correlation coefficient between the measurements was 0.910.

CONCLUSIONS: Iontophoresis-assisted transepithelial CXL creates a demarcation line that can be visualized with AS-OCT, which seems less easily distinguishable and shallower than in conventional CXL. However, its depth and visualization seems to be more similar to conventional CXL than transepithelial CXL.

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Corneal collagen cross-linking (CXL) is the only conservative treatment currently available to halt or reduce keratoconus progression by improving the biomechanical rigidity of the corneal stroma.[1] Several clinical and laboratory studies have already reported the stiffening effect of conventional (epithelium-off technique) CXL treatment.[2-9] But different techniques have been proposed to improve intrastromal penetration of riboflavin molecules and avoid removing the epithelium.[10-12] Iontophoresis-assisted transepithelial CXL is one of these novel approaches, which enhances the penetration of riboflavin in the corneal stroma using a non-invasive delivery system based on a small electric current.[13-15]

After standard CXL, a corneal stromal demarcation line is detectable on slit-lamp examination as early as 2 weeks at a depth of approximately 300 µm.[16] The corneal stromal demarcation line after conventional CXL can also be detected using confocal microscopy and anterior segment optical coherence tomography (AS-OCT).[17-20] In OCT, this demarcation line is represented by a hyperreflective line within the corneal stroma and has been interpreted as ultrastructural changes on stromal collagen.[18] Depth has been associated with the effectiveness of CXL treatment[18-20] because deeper treatment causes more collagen changes and the cornea is supposed to be stiffer. Nevertheless, this supposition has never been shown directly. Mastropasqua et al.[21] recently suggested that the demarcation line could also be a consequence of a non-specific inflammatory reaction because it has been described in the literature in different corneal pathologies.

The current study evaluates the visualization of this demarcation line after iontophoresis-assisted transepithelial CXL and, when possible, its depth.

PATIENTS AND METHODS

PATIENTS

This prospective, consecutive, single center, non-randomized clinical study included 15 eyes of 12 patients (14 eyes of 11 males and 1 eye of 1 female, with ages ranging from 17.2 to 37.4 years [mean age: 25.75 ± 6.2 years]) with keratoconus. The diagnosis was based on corneal topography by Pentacam (Oculus Optikgeräte, Wetzlar, Germany) and slit-lamp examination, including asymmetric bow-tie pattern (curvature power of the inferior segment is higher than that of the superior segment, with a difference of more than 1.5 D on the 4-mm circle),[20] stromal thinning, conical protrusion of the cornea at the apex, Fleischer ring, Vogt's striae, and anterior stromal scar. Cases were classified according to the Amsler–Krumeich grading system. Inclusion criteria were progressive keratoconus and corneal thickness greater than 400 μm. Exclusion criteria were history of intraocular or corneal surgery, herpetic keratitis, central corneal opacities, pregnancy, lactation, and other cornea or anterior segment pathological signs. Keratoconus was described as progressive when there was an increase in the cone apex keratometry of at least 1.0 diopter (D) in 6 months.

Data obtained from patient records included age, sex, biomicroscopy, funduscopy, and AS-OCT scan results (Spectralis; Heidelberg Engineering, Inc., Carlsbad, CA) at 1 month after CXL. AS-OCT scan measurements were performed by two independent examiners. The following measurements include the total corneal thickness by Pentacam, OCT, and the visualization of a demarcation line and its depth (in μm and in percentage of total corneal thickness).

SURGICAL TECHNIQUE

All procedures were performed under sterile conditions. After topical anesthesia with tetracaine chlorhydrate 1% eye drops (Tetracaine; Novartis Corp., Blagnac, France) was applied, the iontophoresis system was established. It is composed of a power supply, a return electrode (a patch adhered to the patient's forehead), a connection cable, and a corneal applicator (Iontofor CXL; SOOFT Italia S.p.A., Montegiorgio, Italy) that contains negative electrodes and adheres with a weak suction (at least 1 mL) vacuum system. After having verified that the applicator was secured on the cornea, the operator covered the steel grid with 0.1% riboflavin solution that was specifically formulated (Ricrolin+; SOOFT Italia S.P.A.). The power generator was then turned on and "1 mA" power was selected. During the procedure, which took approximately 5 minutes (total current intensity: 5 mA), the power supply software indicated the continuity of the procedure.

The operator ensured that the steel grid remained covered with riboflavin solution for the duration of the procedure, thus maintaining a regular flow of the electrical current. After the iontophoretic procedure, the corneal applicator was removed from the cornea. Ultraviolet-A irradiation was then performed using a commercially available ultraviolet-A optical system (C.B.M. X-Linker Vega 10 mW; CSO, Florence, Italy).

Before treatment, an intended irradiance of 10 mW/cm^2 was calibrated using the ultraviolet-A light meter that is supplied with the ultraviolet-X device. Irradiance was performed for 9 minutes, corresponding to a total surface dose of 5.4 J/cm^2. During ultraviolet-A irradiation, balanced salt solution was applied to maintain corneal epithelium hydration. Postoperative medication included topical tobramycin and dexamethasone drops (Tobradex; Alcon Laboratories, Inc., Fort Worth, TX) three times per day for 3 weeks, cycloplegic eye drops (Isopto Homatropine 1%; Alcon Laboratories, Inc.) two times per day for 2 days, and topical lubricants (Vismed; Chemedica AG, Munich, Germany) every 6 hours for 1 month.

AS-OCT

All scans were performed under the same light conditions. Patients were asked to fixate at the optical target in the system. The high-resolution corneal scan was centered on the corneal reflex (a vertical white line along the center of the cornea) and used to produce an enhanced image of the cornea on the horizontal meridian (0° to 180°). When possible, the corneal stromal demarcation line was identified in this enhanced corneal image wand and its depth was measured using the flap tool as provided by the manufacturer.
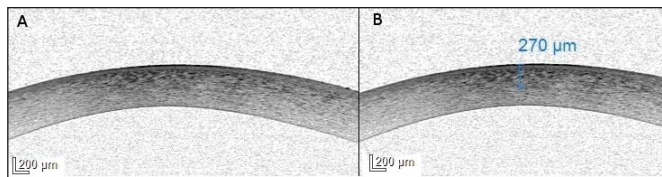
STATISTICAL ANALYSIS

All data were collected in an Excel spreadsheet (Microsoft Corp., Redmond, WA). R-3.0.1 for Windows (R Development Core Team, Vienna, Austria) was used for statistical analysis of the results. Continuous variables are presented as mean ± standard deviation (minimum, maximum) unless otherwise noted. The Pearson correlation coefficient (r) was used to detect relationships between the examiners and stromal demarcation line depths. The agreement between the two examiners was studied using the kappa coefficient method. Normality of distribution of the demarcation line depth was confirmed using the Shapiro–Wilk test when it was identified easily by both examiners. Paired *t* tests with a type 1 error alpha value of 0.05 were then performed between the measurements of the two examiners to determine whether the difference was statistically significant.
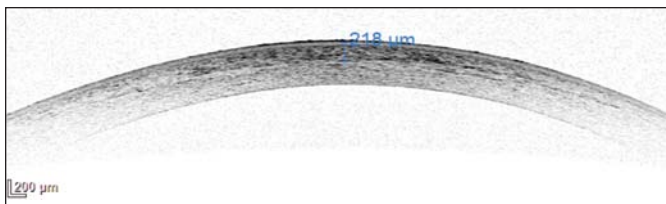
Figure 1. High-resolution corneal anterior segment optical coherence tomography scan visualizing the corneal stromal demarcation line at a depth of 218 μm at 1 month after iontophoresis-assisted transepithelial corneal collagen cross-linking.

Figure 2. High-resolution corneal anterior segment optical coherence tomography scan with a disagreement on visualizing the corneal stromal demarcation line 1 month after iontophoresis-assisted transepithelial corneal collagen cross-linking. The corneal stromal demarcation line is not visualized by the the (A) first examiner, whereas the (B) second examiner noticed a line at a depth of 270 μm.

RESULTS

We observed superficial punctuate keratitis in 6 eyes, but no major intraoperative or postoperative complications (particularly infectious keratitis and ulceration) in any of the patients. The mean central corneal thickness measured with the Pentacam and AS-OCT was 476.5 ± 27.5 µm (range: 428 to 523 µm) and 477.3 ± 30.6 µm (range: 410 to 536 µm), respectively. There was no statistically significant difference between these measurements (paired sample t test, $P > .05$). The visualization (yes or no) and depth of the demarcation line with AS-OCT values (**Figure 1**) for the two examiners are presented in **Table 1**. The corneal stromal demarcation line was identified in 9 eyes (60%) by both examiners. The demarcation line was not visible in 26.7% to 46.7% of eyes, according to the examiners (**Figure 2**). Kappa coefficient estimation for operator agreement in demarcation line visualization was 70.6%. All measurements were reliable for these 9 patients and used in the statistical analysis. Mean depth of the corneal stromal demarcation line was 246.67 ± 50.72 µm (range: 183 to 339 µm) for the first examiner and 241.89 ± 62.52 µm (range: 163 to 358 µm) for the second examiner. There were no statistically significant differences for the measurements for paired comparisons between the two examiners ($P = .61$). The Pearson correlation coefficient of the measurements was 0.910.

DISCUSSION

The exact mechanism of the iontophoresis-assisted riboflavin diffusion is not yet fully elucidated. Several studies have been published with promising results, but none have sufficient long-term evaluation and a large sample

TABLE 1

Score of Visualization and Depth of the Demarcation Line After Iontophoresis-Assisted Transepithelial Corneal Collagen Cross-linking With Anterior Segment Optical Coherence Tomography for the Two Examiners

Eye	Examiner 1		Examiner 2	
	Demarcation Line Visualization (Yes or No)	Demarcation Line Depth, μm (%)	Demarcation Line Visualization (Yes or No)	Demarcation Line Depth, μm (%)
1	Yes	232 (47.9)	Yes	220 (45.5)
2	Yes	183 (44.6)	Yes	163 (39.8)
3	Yes	185 (41.6)	Yes	184 (41.3)
4	No	–	Yes	250 (51.5)
5	No	–	No	–
6	Yes	262 (56.1)	Yes	270 (57.8)
7	Yes	265 (56.7)	Yes	203 (43.5)
8	Yes	297 (59.9)	Yes	301 (60.7)
9	Yes	239 (43.3)	Yes	270 (55.7)
10	No	–	No	–
11	No	–	No	–
12	Yes	339 (64.2)	Yes	358 (67.8)
13	No	–	Yes	270 (57.7)
14	No	–	No	–
15	Yes	218 (47.1)	Yes	208 (44.9)

size to prove the stabilization of the keratoconus. Therefore, the aim of our study was to analyze OCT demarcation line visualization and depth, which have been previously described in conventional CXL and are usually associated with the effectiveness of the CXL treatment.[16]

In our study, the demarcation line was identified in 9 eyes by both examiners, with a kappa coefficient estimation for operator agreement of 70.6%. In a study of 22 eyes with progressive keratoconus treated by iontophoresis-assisted transepithelial CXL, Bikbova and Bikbov[13] observed a demarcation line on Visante OCT (Carl Zeiss Meditec, Inc., Dublin, CA) images in all of their patients. In a study of 6 eyes reporting in vivo riboflavin penetration during iontophoresis-assisted CXL (3 eyes) and conventional epithelium-off CXL (3 eyes) with spectral-domain OCT, Vinciguerra et al.[14] showed a less homogeneous hyperreflective band in the iontophoresis-assisted CXL group than in the conventional CXL group; nevertheless, it was apparent with the same protocol as ours because, unlike Bikbova and Bikbov,[13] we used spectral-domain OCT, which achieves higher speed and resolution than conventional time-domain OCT technology.

Seiler and Hafezi[16] reported that the depth of effective conventional CXL treatment depends on the concentration of riboflavin solution and the intensity of ultraviolet-A light. More recently, Richoz et al.[23] described that oxygen is also an essential element for CXL to occur. Our relatively low rate of demarcation line detection and the less homogeneous hyperreflective band in iontophoresis-assisted transepithelial CXL can probably be explained by those three factors.

Mastropasqua et al.[24] recently detected riboflavin concentration in the anterior, intermediate, and posterior stromas after three CXL imbibition techniques (standard epithelium-off, epithelium-on, and iontophoresis-assisted administration). Their results indicated that iontophoresis-assisted transepithelial CXL yields greater and deeper riboflavin saturation in the anterior cornea (where main CXL effects take place) than transepithelial CXL, but it does not reach the same concentrations obtained with standard CXL. The result on the depth and visualization of the demarcation line in the study by Bikbova and Bikbov[13] is not clearly shown, but it is interesting to note that the total time of riboflavin administration by iontophoresis was 10 minutes, which was twice that in our protocol. In an experimental study on rabbits, Frucht-Pery et al.[25] showed that corneal gentamicin concentration after iontophoresis delivery increases with the current intensity and duration of the iontophoresis.

It can be assumed that the higher intensity used in the study by Bikbova and Bikbov[13] led to a better penetration of the riboflavin in the stroma and greater riboflavin saturation in the anterior corneal stroma, which could explain

the better visualization of the demarcation line in their study. Moreover, in the current study, we used an irradiance of 10 mW/cm² like Vinciguerra et al.[14] but unlike Bikbova and Bikbov,[13] who used an irradiance of 3 mW/cm².

According to the Bunsen–Roscoe law, the effect of a photochemical reaction is directly proportional to the total irradiation dose. However, Wernli et al.[26] and, more recently, Hammer et al.[27] explored the limits of this Bunsen–Roscoe law in porcine corneas after epithelium-off CXL. However, Wernli et al.[26] did not find any difference in corneal stiffening for an intensity lower than 45 mW/cm² and Hammer et al.[27] found a gradual decrease of corneal stiffening with an increase of intensity (starting at 9 mW/cm²). This particular point has not yet been studied in iontophoresis-assisted transepithelial CXL, but it is also possible that the attenuation of the ultraviolet-A radiation by the epithelium enhances the decrease of CXL efficiency described by Hammer et al.[27] and could also explain the better visualization of the demarcation line in the study by Bikbova and Bikbov.[13]

Since the study by Kamaev et al.,[28] the efficiency of CXL is thought to be due to the transformation of oxygen into reactive oxygen species. More recently, Richoz et al.[23] demonstrated in porcine corneas that the biomechanical increase in CXL is oxygen dependent and that oxygen is probably the limiting factor of this reaction. These observations can explain the decrease of efficiency in high-fluence CXL, which cannot allow sufficient time for oxygen to diffuse in the stroma and participate in the reaction. It can also explain the decrease in iontophoresis-assisted transepithelial CXL, in which the intact epithelium can act as a barrier to rapid oxygen diffusion.

In our study, when it was measurable, the mean demarcation line depth was similar to those found in the studies by Bikbova and Bikbov[13] (200 to 250 µm) and Vinciguerra et al.[14] (approximately 200 µm). Seiler and Hafezi[16] reported the depth of the corneal stromal demarcation line after conventional CXL to be approximately 300 µm. The corneal stromal demarcation line was at an average depth of 313 µm for Doors et al.[18] and 281 µm for Yam et al.[19] For Kymionis et al.,[21] the central corneal stromal demarcation line depth was marked as 310.67 ± 31.04 and 308.78 ± 29 µm by the first and the second examiner, respectively, but lesser in nasal and temporal areas. Filippello et al.[10] also saw a demarcation line after transepithelial CXL at a depth of approximately 100 µm, which is considerably shallower than those of the two previous studies. All of these results seem to indicate that the stromal corneal demarcation line depth in iontophoresis-assisted transepithelial CXL is less deep than in standard CXL but deeper than in transepithelial CXL, which is coherent with the lower concentration of riboflavin reported by Mastropasqua et al.[24]

The clinical results obtained with the currently available epithelium-on techniques in treating keratoconus varied.[10,29,30] Even if iontophoresis-assisted transepithelial CXL yields greater riboflavin saturation than transepithelial CXL, it does not reach concentrations obtained with standard CXL and the epithelium may still act as a barrier.

Despite potential limitations, such as the small study sample and absence of a control group, we showed that iontophoresis-assisted transepithelial CXL allows a lesser visualization of the demarcation line in AS-OCT (which seems shallower than in conventional CXL with our protocol), but with an effectiveness more similar to conventional CXL than to transepithelial CXL. Only studies with long-term evaluation and large sample sizes will be able to prove the effectiveness of this new CXL technique.

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