Corneal Collagen Cross-linking in Advanced Keratoconus: A 4-Year Follow-up Study

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ABSTRACT

PURPOSE: To analyze the safety and efficacy of standard corneal collagen cross-linking (CXL) in advanced cases of progressive keratoconus after 4 years of follow-up.

METHODS: A retrospective case series of patients with advanced progressive keratoconus (stages 3 and 4 of Amsler-Krumeich classification) underwent standard CXL treatment. The parameters examined were changes in uncorrected visual acuity (UDVA), corrected visual acuity (CDVA), keratometry values (mean, flat, steep, and apical), pachymetry, and endothelial cell count at the baseline and at 12, 24, and 48 months postoperatively.

RESULTS: Forty eyes of 40 patients were enrolled in the study. The mean patient age was 22.5 years (range: 15 to 37 years). Both mean UDVA and CDVA remained stable during the time points; no statistically significant change was noted. Although a slight reduction was observed in all keratometric readings, a statistically significant reduction was only reached in the apical keratometry ($P = .037$) at 4 years after CXL. A significant reduction in the corneal thickness was also found (ultrasonic: 388 ± 49 to 379 ± 48 µm; slit-scanning: 362 ± 48 to 353 ± 51 µm); however, this change was likely not clinically meaningful. Endothelial cell count was not significantly different at the end of the study. Treatment failure or progression was noted in two patients (5%) over the follow-up period.

CONCLUSIONS: Standard CXL treatment was safe and able to stabilize both visual acuity and topographic parameters at 4 years of follow-up in eyes with advanced keratoconus.

Exposure lasted 2

The procedure was conducted 2

3

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The exposure lasted 2

The procedure was conducted 2

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Paulo, Brazil, and adhered to the tenets of the Declaration of Helsinki. Informed consent was also obtained from all patients after detailed discussion, including alternatives and potential complications. The study was approved by the University of São Paulo and Instituto Oftalmológico Paulista Institutional Review Boards.

Only patients who completed a minimum of 48 months of follow-up after the procedure were included. Inclusion criteria were clinical evidence of progressive keratoconus along with topographic changes of advanced keratoconus.

Advanced keratoconus cases were determined as follows: (1) Amsler-Krumeich stage 3: mean central keratometry reading greater than 53.00 diopters (D) or corneal thickness of 300 to 400 µm or (2) Amsler-Krumeich stage 4: mean central keratometry reading greater than 55.00 D.9

Keratoconus progression was determined based on change in either myopia or astigmatism greater than 1.00 D in the previous 6 months or an increase in maximum keratometry of at least 1.00 D in 1 year. Exclusion criteria were pregnancy or lactation, active anterior segment pathologic features, previous corneal or anterior segment surgery, ocular or systemic disease that could affect the epithelial healing, and dry eye syndrome.

A complete ophthalmologic examination was performed on all patients at the baseline and at 12, 24, and 48 months postoperatively, including uncorrected distance visual acuity (UDVA) and corrected distance visual acuity (CDVA), slit-lamp evaluation, Goldmann applanation tonometry, and dilated fundus examination. In addition, corneal thickness measured by slit-scanning device (Orbscan; Bausch & Lomb, Rochester, NY) and ultrasonic pachymetry (Corneogage Plus; Sonogage, Inc., Cleveland, OH), Placido-based corneal topography (Atlas 9000; Carl Zeiss Meditec, Inc., Dublin, CA), and endothelial cell density using noncontact specular microscopy (Konan Medical, Inc., Hyogo, Japan) were also performed. Parameters evaluated on corneal topography were central 3 mm, simulated keratometry (flat and steep keratometry), mean keratometry, and apical keratometry.

**Standard CXL Technique**

All patients underwent CXL as per the standard Dresden protocol with some variations on type of riboflavin and its presoaking time.3 The procedure was conducted under sterile conditions in the operating room. Briefly, after topical anesthesia, the ocular surface was rinsed with sterile physiologic balanced salt solution and a wire eyelid speculum was applied. The corneal epithelium was manually removed in a central 9-mm diameter area. Before beginning UV-A irradiation, photosensitizing riboflavin 0.1% solution (Ophthalmos, São Paulo, Brazil) was instilled every 2 minutes for 30 minutes to achieve adequate penetration of the solution. Using a slit-lamp with blue filter, the presence of riboflavin in the anterior chamber was confirmed before UV irradiation was started. The cornea was exposed to a UV source emanating from a solid-state device (CSO, Florence, Italy), which emits light at a wavelength of 370 ± 5 nm and an irradiance of 3 mW/cm² or 5.4 J/cm². Exposure lasted for 30 minutes, during which time riboflavin solution was again applied, every 5 minutes. The cropped light beam has a 7.5-mm diameter. A calibrated UV-A meter (LaserMate-Q; Laser 2000, Wessling, Germany) was used before treatment to check the irradiance at a 1-cm distance. Corneas with less than 400 µm were treated with hypotonic riboflavin to swell the cornea up to the 400-µm limit.

Postoperatively, a soft bandage contact lens was worn until reepithelialization was completed. Topical gatifloxacin was given four times daily for 7 days, prednisolone 1% drops three times daily for 20 days, and sodium carboxymethylcellulose 0.5% four to eight times daily as needed.

Stability of keratometric values was defined as postoperative changes within ±1.00 D of the preoperative measurement. Significant flattening was determined as a decrease in keratometry value greater than 1.00 D. Failure of treatment was determined as maximum keratometric progression greater than 1.00 D during the follow-up period. Safety of the procedure was evaluated by loss of Snellen lines in CDVA.

**Statistical Analysis**

Statistical analyses were performed with SPSS for Windows software (version 20.0; SPSS, Inc., Chicago, IL), and all data are reported as mean ± standard deviation. Normality of data was evaluated with the Kolmogorov–Smirnov test. Comparison was performed using generalized estimation equations, first-order autoregressive correlation matrix between moments, normal distribution, and identity link function. Visual acuity was measured using Snellen charts. For statistical analysis purposes, Snellen visual acuity was converted to the corresponding logarithm of the minimum angle of resolution (logMAR) value using standard conversion tables. The level of statistical significance was set at a P value of less than .001 after Bonferroni correction to adjust for multiple comparisons.

**Results**

Forty eyes from 40 consecutive patients, 20 men and 20 women, with a mean age of 22.5 ± 5.3 years (range:
15 to 37 years) were included in the study. Table 1 summarizes the baseline patient data. Mean epithelial healing time after the procedure was 7 ± 2 days. After CXL, a corneal stromal demarcation line was detectable on slit-lamp examination as early as 2 weeks in all eyes.

**VISUAL ACUITY**

The mean baseline logMAR UDVA was 1.17 ± 0.32 (range: 0.6 to 1.6) and remained stable after 48 months of follow-up (1.17 ± 0.31; range: 0.6 to 1.6). No statistically significant change was found ($P = .349$).

The mean preoperative logMAR CDVA was 0.66 ± 0.21 (range: 0.4 to 0.6). The CDVA was 0.66 ± 0.25 (range: 0.3 to 0.6) at 4 years postoperatively. Again, a statistically significant difference was not observed ($P = .363$). Both UDVA and CDVA remained stable after CXL treatment over the follow-up period.

**KERATOMETRIC VALUES**

Analyses of keratometric values revealed that mean values of flattest, steepest, mean, and apical keratometry remained stable or without significant change over 48 months after CXL treatment (Table 2, Figure A, available in the online version of this article). Thirty-eight eyes (95%) presented stable or decreased values of apical keratometry, whereas 2 eyes (5%) showed progression. Among the eyes that remained stable or flattened, 27 eyes (67.5%) had a stable apical keratometry (between 0.00 and 1.00 D), 9 eyes (22.5%) had a flattening of apical keratometry between 1.10 and 2.00 D, and 2 eyes (5%) had decreased keratometry values greater than 2.10 D.

**PACHYMETRY**

The mean central ultrasonic pachymetry readings significantly decreased from 388.20 ± 49.61 to 379.25 ± 48 µm at 48 months of follow-up ($P < .0001$). A significant reduction was also noted in the mean thinnest point measured by the slit-scanning device, which changed from 361.93 ± 47.95 µm preoperatively to 353.30 ± 50.74 µm after 48 months ($P < .0001$).

**ENDOTHELIAL CELL COUNT**

The variation on specular endothelial cell count did not reach statistical significance at any comparison (Table 2). The preoperative mean specular endothelial cell count was 3,005.92 ± 394.98 cells/mm². At 12, 24, and 48 months after CXL treatment, the endothelial cell density was 2,988.02 ± 244.20, 3,038.11 ± 340.80, and 3,007.05 ± 358.01 cells/mm², respectively ($P = .935$).

**SAFETY, FAILURE, AND COMPLICATIONS**

Thirty-five eyes (87.5%) remained unchanged or better, whereas 1 eye (2.5%) lost two lines and 4 eyes (10%) lost one line (Figure 1). Treatment failure or progression was observed in 2 patients. The progression was detected at 12 months of follow-up in 1 patient and at 24 months of follow-up in the other. Postoperative complications included sterile apical keratitis (1 eye) and moderate haze (4 eyes) in the early postoperative stage. No clinically significant haze was observed at 1 year of follow-up.

**DISCUSSION**

Our long-term follow-up study revealed that CXL was safe and effective even in advanced stages of progressive keratoconus. Using these steep keratometry values and relative high curvature as a surrogate for corneal strength measurements, it seems reasonable to assume that these eyes would present lower rates of efficacy. However, we demonstrate that the standard epithelium-off CXL protocol could still be beneficial, effective, and safe over time in eyes with advanced stages of keratoconus. This study is one of the largest samples with the longest follow-up investigating the outcomes of patients who have CXL with stage 3 and 4 Amsler–Krumeich classification.

As opposed to what Koller et al. have stated, although the risk of treatment failure seems to be slightly higher, the literature agrees on the CXL efficacy in this particular group and it is still effective to perform CXL in eyes with maximum keratometry higher than 58.00 D (advanced stages). Our mean preoperative apical keratometry was $58.00 \pm 1.95$ D.
The corneal keratometry value was 64.00 D. Among the 40 eyes with advanced ectatic disease included in our study, the CXL treatment failed in only 2 eyes (5%) after 48 months of follow-up and only 1 eye (2.5%) lost two lines of visual acuity. On the other side, 4 eyes (10%) gained two lines of vision after 48 months.

Both mean UDVA and CDVA remained stable over the follow-up period, representing a benefit in patients with advanced keratoconus because CXL may prevent or at least postpone a corneal transplant. Visual and keratometric stability may also facilitate contact lens use over time.13

Approximately one-third of eyes included in our study presented a flattening in keratometric values higher than 1.00 D at different follow-up time points. As previously shown in the literature,1,11,12,14-16 we were expecting an even more intense remodeling effect in this group comprising eyes with more remarkable signs of topographic abnormality and advanced stages of disease. Corneas that present signs of more severe disease (topographically represented by steeper corneas11,12,14-16) are theoretically more likely to present an intense remodeling effect, with a marked ongoing remodeling effect that could last years after the procedure12 and reaching values up to 14.00 D.12 This effect may vary with the concentration of riboflavin in the stroma and also with the UVA exposure time.17

Previous studies have demonstrated that the combination of increase in regional tissue elastic modulus,18,19 the effective depth of CXL,18 and central cone location19,20 probably explains the localized flattening

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preoperative</th>
<th>12 Months</th>
<th>24 Months</th>
<th>48 Months</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>K flat (D)</td>
<td>53.11 ± 3.4 (47 to 58)</td>
<td>53.00 ± 3.48 (47 to 58)</td>
<td>52.89 ± 3.66 (47 to 59)</td>
<td>52.78 ± 3.75 (47 to 58)</td>
<td>.177</td>
</tr>
<tr>
<td>K steep (D)</td>
<td>59.51 ± 3.92 (50 to 67)</td>
<td>59.36 ± 4.05 (50 to 67)</td>
<td>59.32 ± 4.00 (50 to 67)</td>
<td>59.27 ± 4.09 (50 to 67)</td>
<td>.168</td>
</tr>
<tr>
<td>K mean (D)</td>
<td>56.31 ± 3.57 (49 to 62)</td>
<td>56.18 ± 3.65 (50 to 63)</td>
<td>56.10 ± 3.69 (49 to 63)</td>
<td>56.03 ± 3.79 (49 to 63)</td>
<td>.152</td>
</tr>
<tr>
<td>K apical (D)</td>
<td>64.45 ± 4.59 (56 to 73)</td>
<td>64.44 ± 4.72 (57 to 74)</td>
<td>64.19 ± 4.61 (56 to 72)</td>
<td>63.97 ± 4.92 (56 to 72)</td>
<td>.041</td>
</tr>
<tr>
<td>UDVA (logMAR)</td>
<td>1.17 ± 0.32 (0.60 to 1.60)</td>
<td>1.14 ± 0.32 (0.60 to 1.60)</td>
<td>1.17 ± 0.32 (0.60 to 1.60)</td>
<td>1.17 ± 0.31 (0.60 to 1.60)</td>
<td>.349</td>
</tr>
<tr>
<td>CDVA (logMAR)</td>
<td>0.66 ± 0.21 (0.40 to 1.30)</td>
<td>0.69 ± 0.24 (0.40 to 1.30)</td>
<td>0.66 ± 0.23 (0.40 to 1.30)</td>
<td>0.66 ± 0.25 (0.40 to 1.30)</td>
<td>.363</td>
</tr>
<tr>
<td>Thinnest point (µm)</td>
<td>361.93 ± 47.95 (294 to 459)</td>
<td>354.10 ± 50.27 (249 to 443)</td>
<td>354.62 ± 48.77 (271 to 459)</td>
<td>353.30 ± 50.74 (254 to 459)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>388.20 ± 49.61 (317 to 488)</td>
<td>384.05 ± 49.58 (308 to 485)</td>
<td>384.78 ± 48.02 (301 to 481)</td>
<td>379.25 ± 48.00 (304 to 458)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>ECD (cells/mm²)</td>
<td>3,005.92 ± 394.98 (2,008 to 3,850)</td>
<td>2,988.02 ± 244.20 (2,414 to 3,456)</td>
<td>3,038.11 ± 340.80 (2,297 to 3,875)</td>
<td>3,007.05 ± 358.01 (2,238 to 3,850)</td>
<td>.935</td>
</tr>
</tbody>
</table>

CXL = collagen cross-linking; SD = standard deviation; K = keratometry; D = diopters; mean K = (K1 + K2)/2; apical K = maximum keratometric measurement on topography; UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity; CCT = central corneal thickness; ECD = endothelial cell density

*aStatistically significant change compared to preoperative measurements.

Figure 1. Loss of Snellen lines (%) after 48 months of follow-up. CDVA = corrected distance visual acuity
effect of the cornea. Theoretically, the stiffening associated with the CXL procedure reduces the central corneal strain and shifts the focus of maximum strain toward the limbus, ultimately enabling a central corneal flattening and a hyperopic optical shift.18

Due to cornea irregularity in advanced stages of keratoconus, fewer points are analyzed, which leads to reduced repeatability as a result of keratometry measurement errors and more apparent effect in an advanced group.21 We therefore could not completely rule out that the results understood as stability, in this particular group with advanced stages of keratoconus, could have been the balance between a slight progression that may have been, in fact, compensated for or masked by the flattening effect expected in such a group.

Long-term follow-up studies have shown that when pachymetric changes occur, they tend to be mild.22-25 Although a statistically significant reduction has been achieved, the difference between the mean corneal thickness preoperatively and at 48 months postoperatively was less than 9 µm, which is probably not clinically significant. Early changes in corneal thickness could be related to initial stromal compaction under the effect of the CXL treatment,22 whereas an ongoing thinning process could be associated with the progression, despite the keratometry findings.

Our study revealed no significant change in the endothelial cell count over the follow-up period. The photochemical reaction in general, and the free radicals in particular, produced by the UVA light and riboflavin may be associated with cell death of keratocytes26 and potentially affect the endothelial cells. Although previous studies with longer follow-up periods have demonstrated that CXL is a safe procedure, with no significant endothelial cell damage, keratocyte apoptosis has been documented as deep as 350 µm.27 Additionally, eyes with advanced cases and thinner corneas may be at higher risk of increased reduction of endothelial cells.28

The current study revealed that the standard epithelium-off CXL method seems to be safe and effective in halting ectasia progression in eyes with advanced keratoconus. Both visual and topographic parameters remained stable after the procedure. However, further studies are still needed to confirm corneal and visual stability in longer follow-up periods.

AUTHOR CONTRIBUTIONS

Study concept and design (NTG, MVN, MRS); data collection (NTG, MVN, AAAMT, GKM, SJB, RFE, MRS); analysis and interpretation of data (NTG, MVN, MRS); writing the manuscript (NTG, MVN, AAAMT, GKM, SJB, RFE, MRS); critical revision of the manuscript (MRS); supervision (MVN, MRS).

REFERENCES


Figure A. Mean and standard deviation of keratometric values in diopters over the follow-up period.