Riboflavin/Ultraviolet A Corneal Collagen Cross-linking for the Treatment of Keratoconus: Visual Outcomes and Scheimpflug Analysis

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Purpose: To evaluate the safety and efficacy of corneal collagen cross-linking (CXL) by riboflavin/UV light for the treatment of keratoconus.

Methods: This randomized, prospective, and comparative study involved 10 eyes with keratoconus diagnosed between September 2006 and January 2008. Each patient underwent CXL in the keratoconus eye. Preoperative and postoperative (at 1, 3, 6, and 12 months) biomicroscopy examinations, distance uncorrected and best-corrected visual acuities, refractive error, endothelial cell counts, keratometry readings, ultrasound pachymetry, macular thickness, and Scheimpflug analyses were performed and compared.

Results: Mean uncorrected visual acuity was 1.18 logarithm of the minimum angle of resolution preoperatively and 0.46 logarithm of the minimum angle of resolution at 12 months postoperatively ($P < 0.001$). Statistically significant reductions in the mean maximum [2.66 diopter (D), $P = 0.04$] and minimum (1.61 D, $P = 0.03$) keratometry values were present at 12 months postoperatively, in addition there was a 2.25 D reduction in the mean spherical equivalent ($P = 0.01$). At the end of follow-up, 8 (80%) and 6 (60%) of the 10 eyes showed a decrease in the anterior and posterior elevation values, respectively, and the thinnest point of the cornea was statistically thinner by a mean of 13.4 μm ($P = 0.03$). No statistically significant differences were found between preoperative and postoperative endothelial cell counts and macular thicknesses. The improvements in visual acuity, keratometry readings, and spherical equivalent values occurred progressively during follow-up.

Conclusions: CXL procedure is a safe treatment for keratoconus, yields good visual results, and reduces the progression of the disease, but long follow-up is necessary.

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Keratoconus is a bilateral noninflammatory corneal ectasia.[1] It represents a disorder of the corneal stroma associated with decreased biomechanical strength of the tissue, which is thought to be caused by diminished intra- and interfibrillar cross-links of the collagen fibers.[2]

The use of contact lenses is the most common treatment modality for keratoconus, whereas another option is intrastromal corneal rings.[1–4] In progressive cases that cannot be managed conservatively, a corneal transplant is often required.[4–6]

Corneal collagen cross-linking with the photosensitizer riboflavin and UV light (365 nm) (UV-A) is a method for the treatment of progressive keratoconus. The procedure has been shown to result in increased stiffness of the cornea,[7–11] improved biomechanical strength,[7–11] keratocyte apoptosis,[7] and increased resistance to enzymatic digestion.[11,12] The main aim of this study was to assess the safety and efficacy of corneal collagen cross-linking with riboflavin and UV-A for the treatment of progressive keratoconus.

PATIENTS AND METHODS

This prospective cohort study involved 10 eyes of 10 patients with progressive keratoconus diagnosed at the Oftalmo Salud Instituto de Ojos in Lima, Peru, between September 2006 and January 2008. Inclusion criteria were the diagnosis of keratoconus (Amsler–Krumeich grades I and II), no corneal opacities or scarring on slit-lamp examination, central corneal thickness (CCT) greater than 450 μm (measured by ultrasound pachymetry), and contact lens intolerance (defined as a comfortable wearing time of less than 8 hours per day). Progression was defined by an increase in maximum keratometry of 1.00 diopter (D) in 1 year, patient reports of deteriorating visual acuity (excluding other possible non–cornea-related reasons for deterioration), or a need for new contact lens fitting more than once in 2 years. Exclusion criteria were any other previous or current treatment (s) for keratoconus except contact lenses and an inability to understand the nature of the study or provide informed consent. With regard to the other eye of each patient, 8 of the 10 patients (80%) had

been previously treated for keratoconus (6 had corneal intrastromal rings and 2 had penetrating keratoplasty) and 2 (20%) did not have keratoconus in the other eye at the time of the study.

Each patient underwent corneal collagen cross-linking with riboflavin and UV-A in the keratoconus eye (procedure described in the section Cross-linking Procedure). The following examinations were performed pre- and postoperatively (at 1, 3, 6, and 12 months): distance uncorrected and best-corrected visual acuities (UCVA and BCVA, respectively), manifest refraction, biomicroscopy examination, endothelial cell count (noncontact specular microscope; Topcon, Corp, Tokyo, Japan), maximum and minimum keramometry measurements (Keratron Scout; OPTIKON 2000, Rome, Italy), CCT (Ophthasonic A-Scan/Pachometer III; Accutome, Malvern, PA), corneal thickness at the thinnest point, apex of the keratoconus and pupil center, anterior and posterior elevation values (Pentacam; Oculus Optikgeräte GmbH), and macular thickness (Stratus OCT—optical coherence tomography; Carl Zeiss Meditec, Inc, Jena, Germany) with the fast macular thickness scan.

The control group comprised 10 eyes of volunteers with progressive keratoconus who were recruited from the outpatient clinic. None of the control eyes were receiving any treatment other than glasses. Table 1 shows the visual acuity, refraction, and keratometry of the control eyes at the beginning and end of the study.

The study was approved by the ethics committee of Oftalmo Salud Instituto de Ojos under the principles of the Helsinki Declaration. Informed consent was obtained from all study participants.

Statistical analyses were performed with SPSS (version 12). Comparisons of means were performed using the Student *t* test. Normality of the data distribution was evaluated using the Kolmogorov–Smirnov test. The χ^2 test was used to evaluate proportional differences between follow-ups.

Preparation of 0.1% Riboflavin Solution

Dilute vitamin B2riboflavin-5-phosphate 0.5% (G. Streuli & Co, Uznach, Switzerland) mixed with dextran T500 (Roth AG, Karlsruhe, Germany) was used to create a 0.1% riboflavin solution. The solution was protected from light and used within 24 hours.

Cross-linking Procedure

Topical anesthesia was achieved by instilling 1 drop of proparacaine hydrochloride 0.5% (Alcaine; Alcon Laboratories) into the eye every 5 minutes for 3 doses immediately before the procedure. After positioning the patient under the operating microscope, a lid speculum was inserted and the central 9 mm of the corneal epithelium was removed with a blunt spatula (Asico AE2766). The riboflavin solution was instilled every 5 minutes for 30 minutes until the riboflavin penetrated the cornea as shown by a yellow coloration in the anterior chamber on slit-lamp biomicroscopy. The required irradiance from the UV lamp (UV-X illumination system, version 1000; IROC AG, Zurich, Switzerland) of 3.0 mW/cm^2 was calibrated using a UV-A meter (LaserMate-Q; LASER 2000, Wessling, Germany) at a working distance of 5 cm. The UV radiation was then focused on the apex of the cornea at a distance of 5 cm for a total of 30 minutes, providing radiant energy of 3.0 $\pm$ 0.3 mW/cm^2. During the UV-A administration, the riboflavin solution was applied to the cornea every 5 minutes or sooner if the corneal surface appeared visibly dry.

Postoperative Care

After treatment, the eye surface was washed with 5 mL of balanced salt solution. Two drops of ofloxacin (Oflox; Allergan Laboratories) were instilled, followed by the placement of a bandage soft contact lens (Schalkon SofClear +0.5 D).

Postoperatively, patients received acetaminophen 500 mg twice daily for 3 days, ofloxacin 1 drop 6 times per day for 7 days, and ketorolac tromethamine 0.5% (Acular;

TABLE 1. UCVA, BCVA, Refraction, and Keratometry Values at the Beginning and End of Follow-up for Each Control Eye

	Patient									
Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
UCVA, logMAR*	1.30	1.30	0.30	0.30	0.54	0.54	0.54	0.70	2.00	0.30
UCVA, logMAR†	2.00	2.00	0.48	0.48	0.70	0.54	0.60	0.70	2.00	0.30
BCVA, logMAR*	0.30	0.18	0.10	0.00	0.18	0.10	0.54	0.30	0.70	0.10
BCVA, logMAR†	0.30	0.18	0.30	0.10	1.00	0.18	0.60	0.30	1.00	0.10
K1, D*	44.38	44.28	52.70	50.60	48.91	49.27	52.80	48.20	52.0	43.10
K1, D†	46.77	45.84	53.10	50.30	51.46	49.32	53.70	49.10	65.50	43.20
K2, D*	43.77	43.09	45.90	43.90	43.38	44.76	44.60	40.70	45.70	41.40
K2, D†	42.50	41.99	45.10	43.70	50.59	42.96	45.20	41.20	61.10	41.10
Sphere, D*	−4.50	−4.50	−1.50	0.00	0.00	−3.00	−0.75	−0.75	−5.00	0.50
Sphere, D†	−6.00	−6.25	0.25	0.00	−0.50	−3.00	−2.00	−1.25	−5.50	−1.00
Cylinder, D*	−1.00	−2.00	−1.00	−1.75	−5.00	−4.25	−6.00	−6.25	−6.00	0.50
Cylinder, D†	−0.75	−1.75	−4.00	−2.00	−5.50	−4.75	−6.00	−6.25	−7.00	−1.00

*Parameter evaluated at the beginning of the study.
†Parameter evaluated at 12-month follow-up.
K1, maximum keratometry measurement; K2, minimum keratometry measurement; logMAR, logarithm of the minimum angle of resolution; D, diopters.

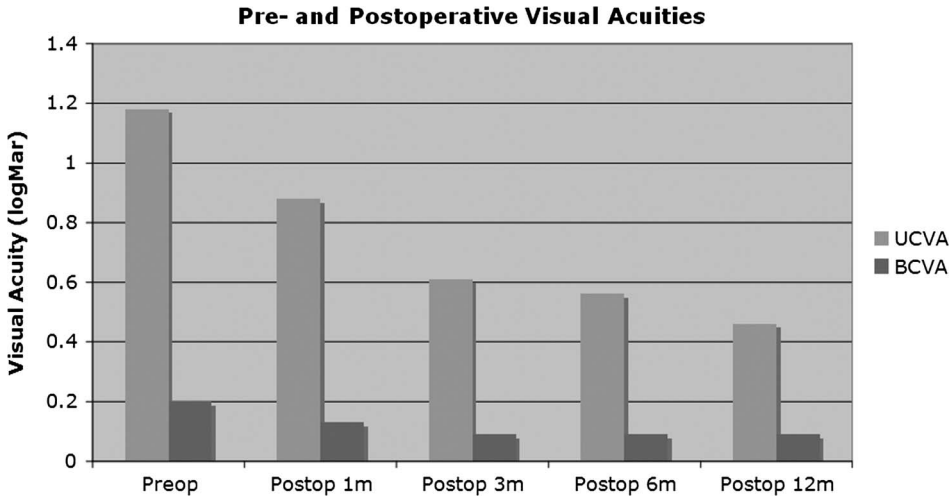

FIGURE 1. Pre- and postoperative UCVA and BCVA. Preop, preoperative visit; postop, postoperative visit; m, month.

Allergan Laboratories) 1 drop 4 times a day for 5 days, followed by fluorometholone (FML; Allergan) twice daily for 5 weeks starting at postoperative day 5. The bandage contact lens was removed on postoperative day 5, at which point slit-lamp biomicroscopy examination was performed to confirm the presence of complete corneal reepithelialization. Routine examinations were performed at 1, 3, 6, and 12 months postoperatively as described previously.

RESULTS

Demographics

The mean age of the patients was 29.7 years (SD: 14.3; range: 15–43 years). Four (40%) of the patients were 25 years old or younger, 1 (10%) was between 25 and 30 years, and 4 (40%) were between 30 and 35 years. One patient was 43 years old; we included him in the study because he had progressive keratoconus diagnoses and all the inclusion criteria. Eight patients (80%) were men and 2 (20%) women.

Visual Acuity

The mean preoperative UCVA was 1.18 logarithm of the minimum angle of resolution (logMAR) (SD: 0.80). Postoperative UCVA at 1, 3, 6, and 12 months was 0.88 (SD: 0.72), 0.61 (SD: 0.46), 0.56 (SD: 0.44), and 0.46 logMAR (SD: 0.36), respectively (Fig. 1). Pre- and postoperative UCVA differences were statistically significant at 3 months ($P = 0.01$), 6 months ($P < 0.001$), and 12 months ($P < 0.001$). Compared with the preoperative UCVA, a postoperative UCVA gain of 1 or more lines was found in 5 eyes (50%) at 1 month, 8 eyes (80%) at 3 months, 9 eyes (90%) at 6 months, and all 10 eyes (100%) at 12 months. In the control group, 6 (60%) of the eyes had worsened UCVA at 12-month follow-up. Table 1 shows the course of the control eyes at the beginning and the end of the study.

The mean preoperative BCVA was 0.20 logMAR (SD: 0.18). The mean postoperative BCVA at 1 month was 0.13 logMAR (SD: 0.12), whereas at 3, 6, and 12 months postoperatively, the mean BCVAs were all 0.09 logMAR (SD: 0.09) (Fig. 1). The differences between pre- and postoperative BCVA values were all borderline significant ($P = 0.07$ for 1 month and $P = 0.06$ for 3, 6, and 12 months). BCVA was unchanged or improved in all eyes compared with the preoperative levels. A postoperative BCVA gain of 1 or more lines of visual acuity was found in 4 eyes (40%) at 1 month and in 6 eyes (60%) at 3, 6, and 12 months. Table 2 lists the UCVA and BCVA over time for each eye included in the study.

TABLE 2. Pre- and Postoperative UCVA and BCVA for Each Study Eye

	Preop UCVA	Postoperative UCVA (logMAR)			
Patient	(logMAR)	1 Month	3 Months	6 Months	12 Months
1	2.00	0.70	0.60	0.60	0.60
2	1.30	1.30	1.30	1.00	1.00
3	0.10	0.00	0.00	0.00	0.00
4	0.60	0.40	0.30	0.30	0.30
5	2.00	0.70	0.48	0.48	0.30
6	2.00	2.00	0.88	0.88	0.88
7	2.00	2.00	0.88	0.88	0.88
8	0.40	0.30	0.30	0.10	0.10
9	1.30	1.30	1.30	1.30	0.48
10	0.18	0.18	0.10	0.10	0.10
	Preop BCVA	Postoperative BCVA (logMAR)			
Patient	(logMAR)	1 Month	3 Months	6 Months	12 Months
1	0.48	0.18	0.00	0.00	0.00
2	0.10	0.10	0.10	0.10	0.1
3	0.00	0.00	0.00	0.00	0.00
4	0.30	0.30	0.18	0.18	0.18
5	0.18	0.10	0.10	0.10	0.10
6	0.10	0.10	0.10	0.10	0.10
7	0.10	0.10	0.10	0.10	0.10
8	0.10	0.10	0.10	0.10	0.10
9	0.54	0.40	0.30	0.30	0.30
10	0.10	0.00	0.00	0.00	0.00

Preop, preoperative.

TABLE 3. Preoperative and 12-Month Postoperative Keratometry Measurements for Each Study Eye

Patient	Preop Kl (D)	Postop Kl (D)	Preop K2 (D)	Postop K2 (D)
1	49.23	49.60	43.83	43.68
2	44.47	45.74	42.78	43.49
3	44.35	44.11	42.40	42.07
4	53.07	49.30	43.77	42.89
5	52.58	43.66	45.86	39.75
6	42.37	41.27	41.11	38.90
7	42.97	41.85	41.35	40.50
8	47.51	44.41	45.55	43.89
9	52.37	43.21	43.28	38.97
10	45.57	44.67	44.14	43.84

D, diopters; K1, maximum keratometry measurement; K2, minimum keratometry measurement; preop, preoperative visit; postop, 12-month postoperative visit.

Keratometry

The mean maximum and minimum keratometry measurements, compared with preoperative levels, were reduced, respectively, by 0.76 and 0.34 D at 1 month postoperatively, 1.00 and 0.94 D at 3 months postoperatively, and 1.39 and 1.01 D at 6 months postoperatively. None of these differences were statistically significant. At 12 months postoperatively, however, the mean maximum and minimum keratometry readings were reduced by 2.66 (P = 0.04) and 1.61 D (P = 0.03), respectively, which did achieve statistical significance. Eight (80%) of the eyes had reductions in the maximum keratometry value, 2 (20%) had worsening of this value, and only in 1 eye was the worsening greater than 1 D. Compared with the control group, 9 (90%) of the eyes had an increase in the maximum keratometry value, and 4 of those had an increase greater than 1 D. Table 3 lists preoperative and 12-month postoperative keratometry measurements for each of the study eyes.

Refraction

Figure 2 shows the mean preoperative and 12-month postoperative refractive errors. There were nonsignificant differences between the pre- and postoperative mean refractive spheres at 1, 3, 6, and 12 months and the mean refractive cylinders at 1, 3, and 6 months. At 12 months postoperatively, the mean refractive cylinder had decreased significantly by 2.25 D (P = 0.02). The spherical equivalent values improved from −4.57 D (SD: 3.55) preoperatively to −3.72 D (SD: 3.03) at 1 month (P = 0.29), −3.66 D (SD: 2.62) at 3 months postoperatively (P = 0.45), and −3.11 D (SD: 2.70) at 6 months postoperatively (P = 0.36). At 12 months postoperatively, there was a statistically significant difference (P = 0.01) with a spherical equivalent of −2.32 D (SD: 2.08).

Pentacam Scheimpflug Analysis

Mean corneal thickness values measured using Scheimpflug analysis with the Pentacam at the thinnest point, pupil center, and apex of the keratoconus are shown in Figure 3. The mean reductions at the thinnest point of the cornea at 1, 3, 6 and 12 months postoperatively compared with preoperative values were 56.2 (P = 0.03), 53.9 (P = 0.01), 26.5 (P = 0.02), and 13.4 μm (P = 0.03), respectively, all of which were statistically significant. There were no statistically significant differences between pre- and postoperative corneal thickness values at the apex of the keratoconus or pupil center.

Table 4 shows the mean pre- and postoperative anterior and posterior elevation values of the cornea. None of the differences between the mean pre- and postoperative values reached statistical significance. Compared with the preoperative values, at 12 months postoperatively, 8 eyes (80%) had a decrease in the anterior surface value (range: 1–19 μm), whereas 6 eyes (60%) had a decrease in the posterior surface value (range: 1–17 μm).

Macular Thickness, Endothelial Cell Count, and CCT

Macular thickness, endothelial cell count, and CCT (measured by ultrasound pachymetry) measurements did not show any statistically significant differences between mean pre- and postoperative (1, 3, 6, and 12 months) levels (Table 5).

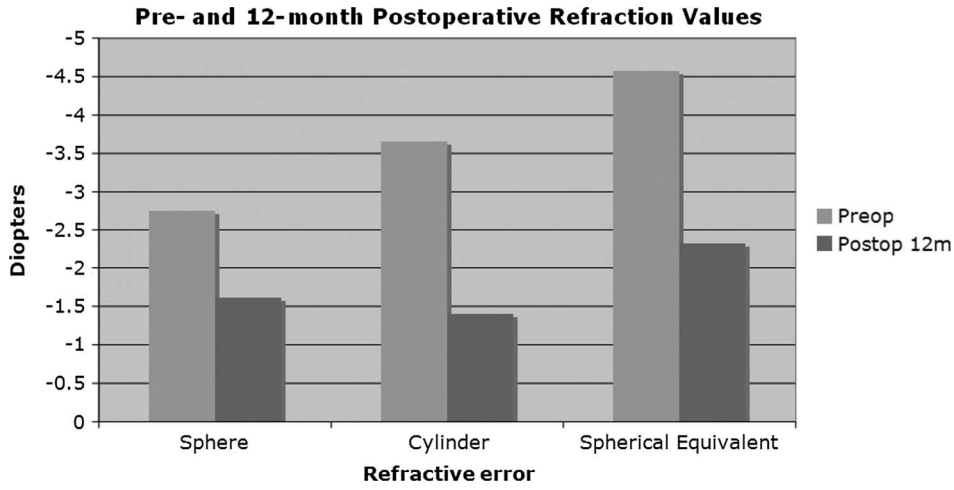

FIGURE 2. Preoperative and 12-month postoperative mean refractive spheres, cylinders, and spherical equivalents. Preop, preoperative; postop 12m, 12 months postoperatively.

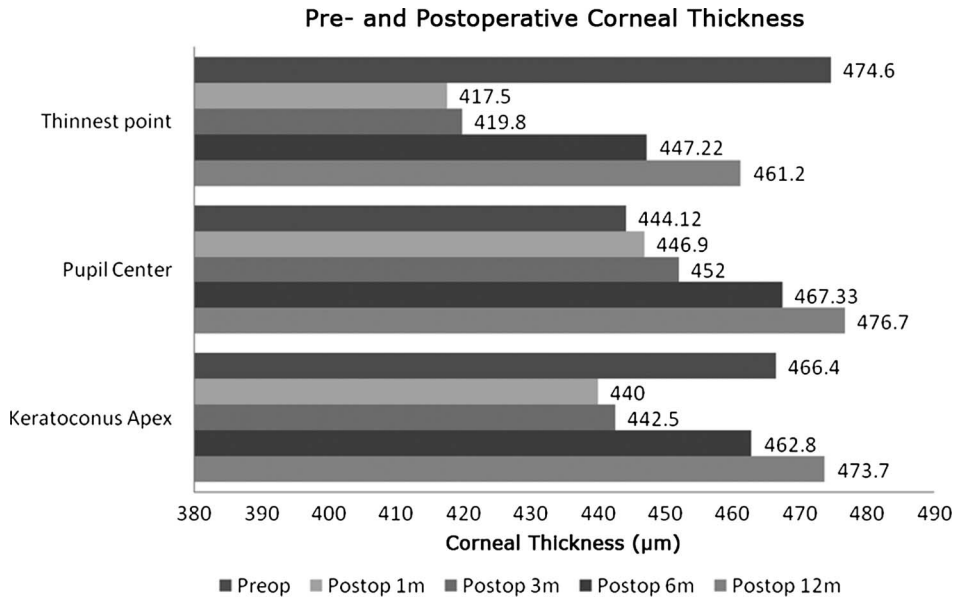

FIGURE 3. Mean corneal thickness at the thinnest point of the cornea, pupil center, and apex of the keratoconus. Preop, preoperative; postop, postoperative; m, month.

Adverse Effects and Postoperative Complications

No intraoperative or serious postoperative complications occurred in this series of patients. One eye presented 1 day postoperatively with Descemet folds and corneal edema, which resolved after 10 days of topical corticosteroid treatment (prednisolone sodium phosphate 5 times per day, Ak-Pred; Akorn).

DISCUSSION

This study demonstrated improved refractive outcomes for patients undergoing riboflavin/UV-A cross-linking treatment for keratoconus. The spherical equivalent value was reduced by a mean of 2.25 D at 1 year postoperatively. Similar refractive results after riboflavin/UV-A cross-linking were reported by Caporossi et al,[13] who found a decrease in the mean spherical equivalent value of 2.21 D at 3 months postoperatively, and by Wollensak et al,[14] who reported that the spherical equivalent improved significantly by an average of 1.14 D at 6 months postoperatively.

With respect to corneal curvatures, we found significant reductions in the mean maximum and minimum keratometry values at 1 year postoperatively of 2.66 and 1.61 D, respectively. Wollensak et al,[14] reported similar results, with a postoperative average reduction of the mean keratometry measurement of 2.01 D. Caporossi et al[13] similarly reported a reduction in the mean keratometry reading of 2.1 D in the central 3.0 mm.

There is little evidence in the literature defining what constitutes normal or abnormal anterior or posterior corneal elevation values. Studies using Pentacam,[15] however, do agree that anterior and posterior corneal curvatures of the cornea are different in normal and keratoconus eyes, with larger curvature values in eyes with keratoconus. In our study, 8 (80%) of the eyes had a reduction in the anterior elevation value and 6 (60%) had a reduction in the posterior elevation value at 1 year postoperatively.

Analysis of the corneal thickness at keratoconus apex or pupil center of the cornea found no statistically significant

TABLE 4. Pre- and 12-Month Postoperative Anterior and Posterior Elevation Values for Each Study Eye

	Anterior Elevation Values (µm)		Posterior Elevation Values (µm)	
Patient	Preop	Postop (12m)	Preop	Postop (12m)
1	9	12	10	21
2	10	9	19	22
3	4	4	14	12
4	14	3	19	5
5	18	10	27	25
6	−2	−7	−4	12
7	2	0	4	7
8	10	9	13	12
9	20	1	33	16
10	6	5	11	10

Preop, preoperative; postop 12m, 12 months postoperatively.

TABLE 5. Pre- and Postoperative Mean CCT, Endothelial Cell Count and Macular Thickness

		Postoperative			
Parameters	Preoperative	1 Month	3 Months	6 Months	12 Months
US pachymetry, µm	471.5	466.5	462.6	462.8	462.8
Endothelial cell count, cell/mm^2	2565.7	2552.8	2385.1	2464.4	2483.5
OCT scan, µm	216	191	191.3	198	200.3

OCT, optical coherence tomography; US, ultrasound.

differences between pre- and postoperative measurements. However, a mean reduction of 56.2 μm in thickness at the thinnest point of the cornea was found at 1 month postoperatively ($P = 0.03$). Although a thinner cornea is not desirable in a keratoconus eye, the amount of thinning at this thinnest point was not progressive and showed improvement over postoperative time, 53.9 ($P = 0.01$), 26.5 ($P = 0.02$), and 13.4 μm ($P = 0.03$), at 3, 6, and 12 months, respectively.

Statistical analysis of the endothelial cell counts and macular thicknesses did not show significant differences between pre- and postoperative data at all follow-up points. These results are consistent with the published literature describing this procedure as safe for the corneal endothelium, as long as the inclusion criteria are met. It is important to have pretreatment CCT of at least 450 μm.[13,14] It is important to have pretreatment CCT of at least 450 m,[13,14] including the epithelium, which is approximately 50-m.[16] Wollensak et al[17] suggested that in human corneas with a CCT less than 400 μm, the cytotoxic endothelial UV-A irradiance of 0.36 mW/cm^2 is reached using the standard treatment surface irradiance of 3.0 mW/cm^2. In our study, all the subjects had CCT greater than or equal to 450 μm, and we did not encounter any serious complications.

It seems logical that the statistically significant reductions in refractive error and keratometry measurements in our study led to the improvements in UCVA. We also believe that the reduction observed in the majority of the eyes in the anterior and posterior corneal elevation values likely contributed to the improved postoperative refractive outcomes.

The improvements in visual acuity, refractive error, and keratometry readings were found to occur progressively over the postoperative follow-up period, suggesting that a longer follow-up is required to achieve the total functional and anatomic effects of the cross-linking treatment and to obtain stable refractive and keratometric values. Once these results are achieved, complementary refractive treatment options such as intrastromal corneal rings may be considered.

In conclusion, this study demonstrated that riboflavin/UV-A cross-linking is likely a safe and effective treatment option for improving visual outcomes and reducing the progression of keratoconus.

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