

Intra- and Postoperative Variation in Ocular Response Analyzer Parameters in Keratoconic Eyes After Corneal Cross-linking

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ABSTRACT

PURPOSE: To analyze intra- and postoperative variation in Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments) parameters in 24 keratoconic eyes undergoing corneal cross-linking (CXL).

METHODS: In a prospective clinical study, corneal hysteresis (CH), corneal resistance factor (CRF), peak 1 and peak 2 amplitude, corneal-compensated and Goldmann-correlated intraocular pressure (IOP) were evaluated using the ORA. The thinnest cornea point was measured with the Pentacam (Oculus Inc). Corneal topography and endothelial cell count were performed. Measurements were recorded at baseline; intraoperatively after epithelium removal, riboflavin impregnation, and ultraviolet A irradiation; and postoperatively after corneal re-epithelialization and at 1, 6, and 12 months.

RESULTS: A statistically significant reduction of the thinnest cornea point from $462 \pm 23.24 \mu\text{m}$ was observed at the end of the CXL procedure intraoperatively and at 1- and 6-month follow-up ($P < .05$). A significant increase in the thinnest cornea point to $624 \pm 31.72 \mu\text{m}$ was found after re-epithelialization ($P < .05$), and no significant changes were observed at 1 year postoperatively. Mean CH and CRF did not change significantly after de-epithelialization, but were noted to significantly increase after CXL intraoperatively and postoperatively at 1-month follow-up. At 6 and 12 months postoperatively, CH and CRF were not statistically significantly different from preoperatively. Peak 1 and peak 2 decreased intraoperatively from 276 ± 52 and 228 ± 47 to 172 ± 42 and 131 ± 42 , respectively, at the conclusion of CXL ($P < .05$), and were noted to increase to 493 ± 41 and 444 ± 51 , respectively, at 6-month follow-up. Corneal-compensated IOP and Goldmann-correlated IOP increased at 1 month after CXL ($P > .05$).

CONCLUSIONS: The results showed a significant change in ORA parameters and the thinnest cornea point during and after the CXL procedure and a high correlation between peak amplitudes and corneal asymmetry, providing insight to the bioelastic and biomechanical behavior of the cornea during and after CXL. [*J Refract Surg.* 2010;26(9):669-676.]
doi:10.3928/1081597X-20100331-01

Keratoconus is a progressive, noninflammatory dystrophy of the cornea of unknown pathogenesis,¹ characterized by a number of histopathologic abnormalities, which lead to a progressive mechanical strength reduction of the cornea over time.²⁻⁴

Preliminary clinical studies have assessed keratoconic corneal biomechanical weakness with the Reichert Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Buffalo, New York), the first simple device able to provide an in vivo dynamic measurement of corneal viscoelastic behavior.⁵ Several studies^{6,7} have shown a significant reduction of two of the parameters measured by the ORA, corneal hysteresis (CH) and corneal resistance factor (CRF), which are significantly lower in keratoconic eyes compared with normal eyes.

Ultraviolet A (UVA) corneal collagen cross-linking (CXL) has been demonstrated to be a safe and effective procedure to definitively halt and stabilize^{8,9} the evolution of keratoconus with a long-term increase in corneal biomechanical rigidity by stiffening the human cornea by approximately 300%,¹⁰ increasing the collagen fiber diameter by 12.2%,¹¹ and the formation of high molecular weight collagen polymers, with a remarkable chemical stability.¹²

The aim of this study was to compare and analyze pre-, intra-, and postoperative biomechanical behavior of 24 eyes with progressive advanced keratoconus undergoing CXL to assess whether the keratoconus stability observed after CXL was associated with a variation of corneal biomechanical parameters measured by the ORA.

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The authors have no proprietary or financial interest in the materials presented herein.

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Received: January 22, 2009; Accepted: February 9, 2010

Posted online: April 15, 2010

PATIENTS AND METHODS

STUDY POPULATION

Twenty-four eyes of 15 consecutive patients (4 females, 11 males) in whom keratoconus progression was detected in the preceding 6 months were enrolled at the Cornea Service of the Ophthalmology Department, Istituto Clinico Humanitas (Rozzano, Milan, Italy) from March to June 2007 in this prospective, nonrandomized, single-center study.

Preoperative keratoconus progression was confirmed by serial differential corneal topographies and by differential optical pachymetry analysis of all eyes included in the study.¹³ The Amsler-Krumeich classification was used for grading keratoconus.¹⁴⁻¹⁷

Inclusion criteria were documented keratoconus progression in the previous 6 months, corneal thickness of at least 400 μm at the thinnest point, and age 14 to 60 years.^{10,18-21} Exclusion criteria included a history of herpetic keratitis, severe dry eye, concurrent corneal infections, concomitant autoimmune diseases, and any previous ocular surgery. Also excluded were pregnant or nursing women, patients with central or paracentral opacities, patients with poor compliance, and patients wearing rigid gas permeable lenses for at least 4 weeks before baseline examination.

The study received approval by the ethical committee of Istituto Clinico Humanitas and was conducted according to the ethical standards set in the 1964 Declaration of Helsinki, as revised in 2000. All patients signed informed consent. The consent form was signed by parents of patients younger than 18 years.

Patients were evaluated at baseline, before CXL, intraoperatively, and postoperatively. During CXL, patients were evaluated after corneal epithelium removal, after riboflavin impregnation immediately before UVA irradiation, and after UVA irradiation before contact lens placement. After CXL, patients were evaluated after lens removal and after corneal re-epithelialization at 1, 6, and 12 months.

During the pre- and postoperative examinations, all patients underwent uncorrected distance visual acuity (UDVA) and corrected distance visual acuity (CDVA) assessment, slit-lamp microscopy, Goldmann tonometry, endothelial biomicroscopy (Konan Specular Microscope; Konan Medical Inc, Hyogo, Japan), corneal topography and total aberrometry (Optical Path Difference [OPD] -Scan; NIDEK Co Ltd, Gamagori, Japan), Pentacam optical tomography (Oculus Inc, Lynnwood, Washington), and ORA assessment.

For the intraoperative measurements, the patient was asked to rise and move to the ORA, OPD-Scan, and Pentacam units where images were taken after the in-

stillation of saline drops. After the examinations, the patient returned to the supine position, was draped anew, the eye rinsed with balanced salt solution (BSS), and a lid speculum inserted. The surgeon then proceeded with the treatment.

CORNEAL TOPOGRAPHY

The OPD-Scan was used to supply data on topography. Specifically, it was used to study the 21 Klyce indices provided by the Corneal Navigator Topo-Classifer Map. In keratoconus diagnosis, the Navigator was reported to be more specific and sensitive than the Rabinowitz-McDonnell Test, and also more specific and sensitive than central corneal power >47.20 diopters (D) or inferior-superior asymmetry >1.40 D.²²⁻²⁵

ANTERIOR CHAMBER ANALYSIS

Anterior chamber analysis was performed with the Pentacam HR, a reliable tool to image and measure the anterior segment of the eye using a rotating Scheimpflug camera.^{26,27} The analyses performed with the Pentacam included pachymetry of the thinnest point of the cornea. X- and y-coordinates showed the distance of this point from the corneal apex.

Total corneal volume is calculated in a ring around the apex, using diameters of 10 mm. Anterior chamber volume is calculated by measuring the distances between the back surface of the cornea and the iris-lens plane over a 12-mm diameter.

OCULAR RESPONSE ANALYZER

The ORA acquisition was performed as follows: the patient was seated in front of the machine and was asked to fixate on a green light. A fully automated alignment system positions an air tube to a precise position relative to the apex of the cornea. Once aligned, a 30-ms air pulse applies pressure to the cornea. The air pulse causes the cornea to move inward, past appplanation and into a slight concavity before returning to normal curvature. Corneal appplanation is recorded via an electro-optical infrared detection system (similar to the classical air-puff tonometers).

Each patient's ORA measurement is the mean of four consecutive air-puff appplanations. Irreproducible ORA measures, which occur in cases of abnormal corneal movements or surface irregularities, were excluded from the study by the clinician.

As specified by Touboul et al,²⁸ using a bidirectional appplanation measurement, the ORA is able to present four different parameters. Corneal hysteresis, the difference between inward appplanation amplitude peak 1 and outward appplanation amplitude peak 2, is a function of the corneal viscous-damping properties and is

likely linked to the stromal collagen nature and state of hydration.²⁹ Corneal resistance factor, also a viscoelastic parameter, is calculated using a linear combination of peak 1 and peak 2 and more heavily weighted by the underlying corneal elastic properties.³⁰ Corneal-compensated intraocular pressure (IOP) measurement is strongly correlated to Goldmann tonometry and is also calculated using a specific linear combination of peak 1 and peak 2.³⁰ Corneal-compensated IOP should have little correlation with central corneal thickness measured by ultrasound pachymetry, and it has been reported to remain fairly constant after refractive surgery.²⁹ The amplitude of the peaks (peak 1 and peak 2) is a function of how much light hits the infrared detector during each applanation event. If the applanation area is large, the peak amplitude will be large; if it is small, the peak amplitude will be small. Deformed ectatic corneas seem to have an abnormal signal shape, as described by Kérautret et al.³¹

CROSS-LINKING PROCEDURE

All patients underwent CXL on a same-day surgery basis. Thirty minutes before the procedure, pain medication was administered and 2% pilocarpine drops were instilled in the eye to be treated. Because the amount of light rays reaching the retina is proportional to the square of the pupil diameter, the use of pilocarpine reduces the thermal and photochemical UVA light irradiation potentially harmful to the lens and retina.

The procedure was conducted under sterile conditions in the operating suite. After topical anesthesia with two applications of 4% lidocaine drops and oxybuprocaine hydrochloride 0.2%, the patient was draped, the ocular surface was rinsed with BSS, and a lid speculum inserted. The corneal epithelium was abraded in a central, 9-mm diameter area with the aid of an Amoils brush (Vision Technology Co Ltd, Seoul, Korea).

Before beginning UVA irradiation, photosensitizing riboflavin 0.1% solution (10 mg riboflavin-5-phosphate in 20% dextran-T-500 10 mL solution) was applied to the cornea every minute for 30 minutes to achieve adequate penetration of the solution. Using a slit-lamp with blue filter, the surgeon confirmed the presence of riboflavin in the anterior chamber before UVA irradiation was started. The cornea was exposed to a UV source emanating from a solid-state device (CBM XLinker; C.S.O., 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, this time once every 5 minutes. The cropped light beam has a 7.5-mm diameter. A calibrated UVA meter (LaserMate-Q; Laser 2000, Wessling, Germany) was

used before treatment to check the irradiance at a 5.0-cm distance. Fixation during irradiation was achieved by instructing the patient to focus on the central green light of the probe. During the procedure, centration of treatment was controlled by the surgeon. Both topical anesthetics were added as needed during irradiation.

Postoperatively, patients received cyclopentolate (Ciclolux; Allergan, Rome, Italy) and levofloxacin drops (Oftaquix; Tubilux Pharma, Pomezia RO, Italy). A soft bandage contact lens was applied until re-epithelialization was complete. Topical levofloxacin was given four times daily for 7 days, dexamethasone 21-phosphate 0.15% drops (Etacortilen; Sifi, Lavinaio CT, Italy) three times daily for 20 days, and 0.15% sodium hyaluronate drops (BluYal; Sooft, Montegiorgio AP, Italy) six times daily for 45 days. In addition, all patients received oral amino acid supplements (Trium, Sooft) for 7 days.³¹ Patients were examined every day until re-epithelialization and then at 1, 6, and 12 months postoperatively.

DATA ANALYSIS

Statistical analyses were performed with the Statistica (StatSoft Inc, Tulsa, Oklahoma) software package. All data are reported as mean \pm standard deviation. Normality of the data was tested using the Kolmogorov-Smirnov test and the normal probability plot.

Correlation between peak 1, peak 2, simulated keratometry, apical corneal power, and surface asymmetry index were checked between baseline, intra-, and postoperative examinations. Relationships between parameters with an analysis-of-variance *P* value $< .05$ and a Pearson coefficient (*r*) > 0.65 were considered to be highly correlated. The level of statistical significance within each group (paired Student *t* test) was set at $P < .05$.

RESULTS

Patient age ranged from 15 to 36 years. All treated eyes were graded stage II according to the Amsler-Krumeich classification. Mean re-epithelialization time was 43 ± 8 hours. Follow-up was 12 months for all patients included in the study. Tables 1 and 2 show the statistically significant topographic, tomographic, and biomechanical changes that occurred before, during, and after CXL.

TOPOGRAPHIC RESULTS

Klyce indices obtained with the NIDEK OPD platform were analyzed in all treated eyes at baseline and at 12 months and results are shown in Table 1. At 12 months postoperatively, most of the Klyce indices of the treated eyes had statistically significantly decreased ($P < .05$) compared with preoperative data.

TABLE 1

Klyce Indices Measured With the NIDEK OPD-Scan at Baseline and 1 Year After Corneal Cross-linking in 24 Eyes With Keratoconus

Indices	Mean \pm Standard Deviation		P Value
	Before CXL	1 Year Postop	
Simulated keratometry 1 (D)	51.56 \pm 1.02	50.83 \pm 0.08	<.05
Simulated keratometry 2 (D)	46.73 \pm 2.59	46.58 \pm 2.34	>.05
Minimum keratometry (D)	43.03 \pm 1.8	43.19 \pm 2.4	>.05
Apical corneal power (ACP)	50.02 \pm 1.14	48.67 \pm 0.92	<.05
Simulated keratometry cylinder (CYL)	4.83 \pm 0.76	4.26 \pm 0.82	<.05
Coefficient of variation of corneal power (CVP)	99.10 \pm 9.01	89.40 \pm 6.03	<.05
Standard deviation of corneal power (SDP)	4.81 \pm 0.73	4.31 \pm 0.62	<.05
Analyzed area (AA) (%)	81.79 \pm 1.83	79.27 \pm 0.92	<.05
Corneal eccentricity index (CEI)	1.07 \pm 0.64	1.00 \pm 0.79	>.05
LogMAR	0.26 \pm 0.04	0.23 \pm 0.04	<.05
Differential sector index (DSI)	11.61 \pm 1.51	10.54 \pm 1.23	<.05
Surface regularity index (SRI)	1.67 \pm 0.37	1.58 \pm 0.85	>.05
Area compensated surface regularity index (SRC)	1.45 \pm 0.29	1.41 \pm 0.28	>.05
Surface asymmetry index (SAI)	2.72 \pm 0.41	2.48 \pm 0.36	<.05
Irregular astigmatism index (IAI)	0.62 \pm 0.06	0.61 \pm 0.08	>.05
Opposite sector index (OSI)	9.25 \pm 1.7	8.43 \pm 1.3	<.05
Center surround index (CSI)	2.99 \pm 0.85	2.55 \pm 0.62	<.05
Keratoconus prediction index (KPI)	0.42 \pm 0.37	0.39 \pm 0.27	>.05
Elevation/depression power (EDP)	3.70 \pm 1.54	3.31 \pm 1.62	>.05
Elevation/depression diameter (EDD)	15.84 \pm 2.12	14.37 \pm 1.23	<.05

CXL = cross-linking, logMAR = logarithm of the minimum angle of resolution
 Note. Bold indicates statistical significance (paired Student t test).

As shown in Table 2, mean apical corneal power increased from 50.02 \pm 1.14 D at baseline to 52.64 \pm 1.62 D after corneal epithelium removal ($P=.04$). Riboflavin impregnation and UVA irradiation did not induce a statistically significant further change in apical corneal power ($P>.05$). Apical corneal power was less than the preoperative value at 6 months. At 12 months, apical corneal power decreased to 48.67 \pm 0.92 D, which was statistically significant ($P=.03$) compared to the preoperative value.

TOMOGRAPHIC RESULTS

Corneal pachymetry at the thinnest point measured with the Pentacam decreased from 462 \pm 23.24 μ m at baseline to 430 \pm 26.85 μ m after de-epithelialization ($P=.06$) (Table 2). Riboflavin impregnation and UVA irradiation reduced the thinnest point to 407 \pm 18.21 μ m and 379 \pm 21.57 μ m, respectively, and the difference from preoperative data was statistically significant

($P<.05$). During postoperative follow-up, when the soft bandage contact lens was removed, re-epithelialization was completed and the cornea became less edematous; the thinnest point increased to 624 \pm 31.72 μ m, a difference that was statistically significant ($P=.001$) compared to the preoperative data. From 1 to 6 months postoperatively, the thinnest point was lower than preoperative values, decreasing to 422 \pm 19.12 μ m at 1 month ($P=.03$) and 428 \pm 18.57 μ m at 6 months ($P=.04$). However, at 12 months postoperatively, the cornea regained its thickness and no statistical difference was noted in the thinnest point, which recovered to 451 \pm 22.45 μ m ($P=.07$).

Mean total corneal volume, similar to the thinnest point, decreased from 56.17 \pm 5.23 mm³ at baseline to 54.13 \pm 3.17 mm³ at 1 month postoperatively ($P=.04$). At 6 and 12 months, it recovered to 55.69 \pm 4.88 mm³ and 56.02 \pm 4.67 mm³, respectively, a difference that was not statistically significant ($P>.05$).

TABLE 2
Parameters Measured Pre-, Intra-, and Postoperatively in 24 Eyes With Keratoconus That Underwent Corneal Cross-linking

	Mean ± Standard Deviation							
	Baseline	After De-Epi	After Riboflavin	After UVA Irradiation	After Re-Epi	1 Month	6 Months	12 Months
CH	9.13±1.71	10.08±1.82	14.51±1.52*	15.09±1.43*	11.33±1.42*	12.48±1.46*	10.25±1.62	9.27±1.25
CRF	9.05±1.76	9.42±1.83	13.87±1.48*	14.15±1.32*	10.24±1.44*	12.53±1.53*	10.13±1.52	9.01±1.12
IOPcc	14.62±2.45	13.45±2.53	13.86±2.41	14.04±2.43	12.24±2.18	16.71±2.41	14.06±2.51	14.04±2.44
IOPg	13.03±3.44	12.44±3.26	13.21±3.15	14.41±3.3	15.07±3.16	18.1±3.52	11.01±3.12	12.17±3.15
Peak 1	276±52	247±48	229±57	202±48†	172±42†	326±53	493±41*	439±46*
Peak 2	228±47	187±39	153±65	139±43†	131±42†	287±52	444±51*	346±47*
ACP	50.02±1.14	52.64±1.62	52.85±3.08	52.94±2.71	53.79±2.96	53.54±3.01	49.4±1.07	48.67±0.92
TP (μm)	462±23.24	430±26.85	407±18.21*	379±21.57*	624±31.72†	422±19.12	428±18.57	451±22.45

Epi = epithelialization, CH = corneal hysteresis, CRF = corneal resistance factor, IOPcc = corneal compensated intraocular pressure, IOPg = Goldmann correlated intraocular pressure, ACP = apical corneal power, TP = thinnest point as measured by the Pentacam

*Statistically significant increased values ($P < .05$).

†Statistically significant decreased values ($P < .05$).

No statistically significant changes during 1-year follow-up were observed in anterior chamber volume, anterior chamber depth, and anterior and posterior elevation. Anterior chamber volume changed from $206.39 \pm 24.85 \text{ mm}^3$ at baseline to $198.21 \pm 34.52 \text{ mm}^3$ at 1-year follow-up. Anterior chamber depth decreased from $3.52 \pm 0.18 \text{ mm}$ to $3.43 \pm 0.12 \text{ mm}$, and anterior and posterior elevation, respectively, changed from $6.94 \pm 0.32 \text{ mm}$ and $6.21 \pm 0.44 \text{ mm}$ to $7.02 \pm 0.36 \text{ mm}$ and $6.58 \pm 0.48 \text{ mm}$.

ENDOTHELIAL RESULTS

No statistically significant changes ($P = .06$) were observed in endothelial cell count at 1-year follow-up, indicating that CXL did not induce any endothelial damage in the first postoperative year.

Mean endothelial cell count at baseline was 2832 ± 254 cells/ mm^2 . One month after the procedure it was 2657 ± 620 cells/ mm^2 , at 3 months 2753 ± 580 cells/ mm^2 , at 6 months 2720 ± 562 cells/ mm^2 , and at 12 months endothelial cell count was 2810 ± 352 cells/ mm^2 .

OCULAR RESPONSE ANALYZER

Corneal hysteresis and CRF showed similar behavior during and after CXL (Table 2). Corneal epithelium removal did not affect CH and CRF ($P = .06$). However, riboflavin impregnation and UVA irradiation increased both parameters significantly ($P < .05$). After the procedure, CH and CRF remained statistically significantly higher than the preoperative values only at 1-month follow-up. At 6 and 12 months postoperatively, no statistically significant changes were noted in CH and CRF.

De-epithelialization, riboflavin impregnation, and UVA irradiation significantly reduced peak 1 and peak 2 amplitude ($P < .008$) (Table 2). Both parameters remained significantly low after contact lens removal ($P < .005$). However, the amplitude of peak 1 and peak 2 increased to double the preoperative value at 6 and 12 months postoperatively ($P = .001$).

Statistically significant substantial correlation ($r > -0.65$) was found between peak 1 and peak 2 and simulated keratometry steepest meridian, simulated keratometry flattest meridian, apical corneal power, and surface asymmetry index.

Corneal-compensated IOP and Goldmann-correlated IOP did not change significantly during the CXL procedure. However, a transient increase in IOP was observed at 1-month follow-up ($P = .03$) (Table 2).

DISCUSSION

This is the first study in which the viscoelastic characteristics of keratoconic corneas were studied with the ORA during and after CXL and the first study to report the thickness variations of the cornea during the riboflavin/UVA treatment.

The study showed how corneal de-epithelialization, riboflavin impregnation, and UVA irradiation increased the steepness and asymmetry of keratoconic eyes with a progressive reduction of corneal thickness—peak 1 and peak 2 during the entire procedure. Corneal de-epithelialization did not induce any change in CH and CRF; however, both parameters increased during CXL at the end of the impregnation and irradiation phases.

After CXL, the effects of corneal re-epithelializa-

tion and soft bandage contact lens removal were a decrease in CH and CRF, a reduction of peak 1 and peak 2 amplitudes, and a 15% increase of corneal thickness.

From 1 to 12 months postoperatively, peak 1 and peak 2 amplitude increased progressively, while CH and CRF did not show a statistically significant change when compared with preoperative values, except at 1 month after CXL. At the same time, as shown in Table 1, cone steepness and all indices related to corneal asymmetry tended to progressively decrease compared with preoperative data, confirming the results of several clinical reports.^{8,9} Interestingly, corneal thickness tended to recover slowly 1 year after CXL.

During CXL, corneal de-epithelialization did not seem to influence CH and CRF parameters in keratoconic corneas. Corneal hysteresis increased from 9.13 ± 1.71 at baseline to 10.08 ± 1.82 after de-epithelialization and CRF increased from 9.05 ± 1.76 at baseline to 9.42 ± 1.83 after de-epithelialization—a difference that was not statistically significant for either parameter. This finding shows that the corneal epithelium does not seem to affect the structural stability of the cornea, which differs from the most anterior part of the corneal stroma (100 to 120 μm), which could be responsible for the stability of the corneal shape, as stated by Muller et al.³² In contrast, Touboul et al²⁸ and Gatinel et al³³ have shown that the creation of a superficial stromal flap, which precedes excimer laser ablation, can modify the biomechanical properties of the cornea, with a statistically significant reduction of CH and CRF.

In our study, corneal de-epithelialization influenced the peak amplitudes, which decreased statistically significantly (Table 2). As Kérautret et al³¹ suggested in their study, the peak amplitudes were negatively correlated with keratoconus severity, being an indicator of small applanation area and non-uniform corneal deformation. As shown by the intraoperative topographic analysis, soon after corneal de-epithelialization, apical corneal power increased, the cornea became steeper, the area applanated by the ORA became smaller, and the corneal deformation less uniform with a reduction of peak amplitude. In addition, after corneal de-epithelialization, the surface of the cornea became qualitatively rougher and uneven, which might explain lower signal amplitudes at the ORA examination.

To further understand the increase in CH and CRF during the impregnation and irradiation phases of the CXL procedure, it was important to evaluate the corneal thickness change during the procedure itself. As expected, the thinnest cornea point decreased after de-epithelialization. In addition, we observed a continuous reduction of the thinnest point during the first

and second phase of CXL, with a 15% loss of thickness after the impregnation phase and a 21% loss of thickness after the UVA irradiation phase.

This thickness reduction could be due to the dehydrating effect of T-dextran, which is part of the riboflavin solution. Despite this thickness reduction, we did not observe any harmful effect on the endothelial cell count, which remained stable over 1 year postoperatively. Corneal hysteresis and CRF, which are related to the damping nature of the cornea (eg, collagen structure, hydration state), significantly increased in these two phases. On the contrary, peak amplitudes did not change statistically significantly compared to the de-epithelialized corneal values, probably because the corneal surface was qualitatively rougher and uneven, providing lower signal amplitudes at the ORA examination. These results suggest that the dehydrated cornea was more resistant and stiff.

During the re-epithelialization phase, corneal thickness increased significantly due to the corneal edema observed at the slit lamp a few days after CXL, when the bandage soft contact lens was removed. Corneal hysteresis and CRF decreased but showed no statistically significant difference from the preoperative values, and peak amplitudes remained low. These results suggest that the edematous cornea was less elastic, less resistant, and weak.

After CXL, cone steepness and corneal asymmetry related indices tended to decrease progressively compared with preoperative data. This improvement, however, cannot be expected immediately after CXL, when the corneal topography is evaluated after epithelium removal. As the intraoperative topographic analysis has shown, a statistically significant increase of apical corneal power was noted after de-epithelialization. The cornea became steeper once the epithelium had been removed, meaning that the epithelium was having a compensatory effect on the cone, affecting total corneal power,³⁴ masking the true curvature of keratoconic corneas,³⁵⁻³⁸ and acting as a smoothing agent that fills all of the areas in which the corneal gradient is too high.^{32,39-43}

Within 1 year, the epithelium slowly recovered over the corneal stroma, as well as the values of the thinnest point, which were not statistically significantly different than the preoperative data. Further measurement of corneal epithelial thickness over time with a very high-frequency digital ultrasound scanner is required to assess whether any eventual increase of corneal epithelium thickness after CXL could explain why the flattening effect of CXL can be topographically documented 6 months postoperatively.

With the slow progressive restoration of the corneal thinnest point during the first year of follow-up, we did

not observe a statistically significant change in CH and CRF compared with baseline. However, the increased regularity of the corneal surface, assessed with a reduction of simulated keratometry of the flat and steep meridians, corneal asymmetry, and cone steepness itself (apical corneal power) were all highly correlated with the increase of peak amplitudes, thus demonstrating that the shape of the cornea tended to become more similar to a normal corneal shape and the severity of the disease tended to diminish.

The fact that no statistically significant difference occurred in CH and CRF after CXL compared with baseline suggests that the change in stiffness may be less than that which can be measured by the sensitivity of the ORA, or it might indicate that CXL changed both elasticity and viscosity in a manner that was not detected by the viscoelastic parameters, CH and CRF.⁴⁴ Cross-linking did not induce any effect on IOP, as measured with the ORA. Goldmann-correlated IOP and corneal-compensated IOP did not change significantly over the first postoperative year, except during the first postoperative month, after which the steroid therapy was tapered.

The results of the current study demonstrate that the viscoelastic biomechanical characteristics of keratoconic corneas that underwent CXL were significantly correlated with changes in corneal thickness, hydration, and tissue composition. Peak amplitudes were significantly correlated with corneal asymmetry and irregular corneal shape.

AUTHOR CONTRIBUTIONS

Study concept and design (P.V., E.A.); data collection (P.V., E.A., S.T.); analysis and interpretation of data (P.V., E.A., A.M.M., S.T., F.H., C.J.R.); drafting of the manuscript (P.V., E.A., A.M.M.); critical revision of the manuscript (P.V., E.A., S.T., F.H., C.J.R.); statistical expertise (E.A.); administrative, technical, or material support (S.T.); supervision (P.V.)

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