

ORIGINAL ARTICLE

Intraoperative and postoperative corneal thickness change after collagen crosslinking therapy

Ziad Hassan¹, Laszlo Modis Jr.², Eszter Szalai², Andras Berta², Gabor Nemeth²

¹ Orbident Refractive Surgery and Medical Center

² Department of Ophthalmology, Medical and Health Science Center, University of Debrecen - Hungary

Purpose: To assess intraoperative and postoperative changes in corneal thickness subsequent to riboflavin-UVA (collagen crosslinking [CXL]) treatment.

Methods: Forty-one eyes of 41 patients (mean age 27.97 ± 6.97 years) were treated with CXL technique. During treatment, isotonic riboflavin was instilled and corneal thickness measurements were obtained at the cornea apex, the thinnest point, and the pupil center at 15 and 30 minutes, then 3 days, 1 week, and 1, 3, 6, and 12 months after surgery using Pentacam HR and an ultrasound pachymeter.

Results: A decrease in corneal thickness was detected 15 minutes intraoperatively with a value of $108.95 \pm 48.6 \mu\text{m}$, and $112.35 \pm 47.3 \mu\text{m}$ at 30 minutes ($p < 0.001$). Three days after the operation, no deviation was found from the initial values ($p = 0.17$). No further changes were detected during the follow-up period.

Conclusions: Isotonic riboflavin solution used during CXL treatment resulted in a significant decrease in corneal thickness, but its effect had disappeared by postoperative day 3.

Keywords: Collagen crosslinking, Corneal thickness, Isotonic riboflavin, Pentacam HR

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INTRODUCTION

Collagen crosslinking (CXL) is a promising treatment applied to delay and stop the progression of keratoconus (1-4). On one hand, riboflavin used during treatment acts as a photosensitizer; on the other hand, it protects the corneal endothelium from UVA radiation (5, 6). During and subsequent to this treatment, a significant change in corneal thickness can be detected (7-10), thus the protective effect of riboflavin is likely to fail in some cases. Our goal was to assess the changes in the thickness of corneas treated with isotonic riboflavin during and 1 year after surgery.

PATIENTS AND METHODS

Inclusion criteria for CXL were the following: keratoconus diagnosed on the basis of the known criteria and the

Collaborative Longitudinal Evaluation of Keratoconus Study (11, 12); age 18 to 45 years; cornea thicker than $400 \mu\text{m}$ at the thinnest point; and significant decline in visual acuity and/or keratometric values and corneal pachymetry in the past 3-6 months. Further inclusion criteria were optically clear cornea and a lack of Vogt striae or anterior stromal scarring. Exclusion criteria were dry eye syndrome distorting the images captured by Pentacam HR and eye pathology other than keratoconus.

Prior to operation, the following data were recorded: age, sex, and corneal thickness measured at the apex, the pupil center, and the thinnest point of the cornea using Pentacam HR (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany, software version 1.17r139). Pentacam HR is the high-resolution version of the former Pentacam. Using a monochromatic light source (475 nm) and rotating around the optical axis, the camera captures 25 Scheimpflug images in 2 seconds while also correcting for small eye

movements. For each examination, 3 of these complete images were captured of every eye and only automatically captured images with no distortion (such as blinking) were included. Additionally, ultrasound measurements were carried out at the center of the cornea at each visit, using an ultrasound pachymeter (PachPen; Accutome Inc., Malvern, Pennsylvania, USA).

Surgical technique

Topical anesthesia was performed by administering tetracaine hydrochloride eyedrops 15 minutes before the operation and every 5 minutes until the end of the operation. The patient was placed under the operating microscope and a lid speculum was inserted, the epithelium was marked in the inferior paracentral area to be treated (diameter 8-9 mm), and the epithelium was removed with a blunt spatula. Before starting UVA irradiation, the cornea was instilled with riboflavin 0.1%, dextran 500 20% solution for 15 minutes (single-use isotonic eyedrops; Medio Cross Medizin Produkte GmbH, Rostock, Germany) with a frequency of every 5 minutes for the drops. In cases of corneas with a central corneal thickness (CCT) of under 400 μm , we used hypotonic riboflavin solution (single-use hypotonic eyedrops; Medio Cross Medizin Produkte GmbH) for 1 hour before irradiation to swell the cornea. Then the cornea was exposed to a UVA 370-nm light (In-Pro CCL-Lix UV, Norderstadt, Germany) for 30 minutes at an irradiance of 3.0 mW/cm^2 (5.4 J/cm^2). During UVA exposure, isotonic riboflavin drops were continued every 2 minutes or more often if the corneal surface appeared visibly dry.

After the first 15-minute treatment, the cornea was rinsed with saline to prevent riboflavin altering measurement values and topical anesthetics were used as most patients complain of eye sensitivity at this stage. The demonstrating 15-minute Pentacam HR images were captured at this stage. In one case, intraoperative images were captured also with a Visante optical coherence tomograph (Visante OCT, Carl Zeiss Meditec, Jena, Germany). Then riboflavin UVA treatment was continued for a further 15 minutes with instillation every 2 minutes. After treatment, the eye surface was washed with antibiotic drops, a process that was followed by the placement of a bandage soft contact lens (Air Optix Night and Day, Alcon, Fort Worth, Texas, USA). Postoperatively, antibiot-

ic (3 mg/mL tobramycin) drops 5/day were administered for 5 days, corticosteroid (1 mg/mL fluometholone) drops 5/day for a minimum of 1 month starting at postoperative day 5. The bandage contact lens was removed on postoperative day 3, at which point slit-lamp examination was performed to confirm the presence of complete corneal re-epithelization. Routine examinations were performed 3 days, 1 week, 1 month, 3 months, 6 months, and 12 months postoperatively.

All treatments were carried out by the same experienced operator. All patients were informed about the details of the examinations and the operation and signed an informed consent. All examinations were conducted according to the protocol of the Declaration of Helsinki.

Statistical analysis was performed with MedCalc 10.0. Descriptive statistical results were described as mean, standard deviation (SD), and 95% confidence interval (95% CI) for the mean. A Wilcoxon test was carried out for comparisons between groups or variables, and a Spearman rank test for correlation. A p value below 0.05 was considered statistically significant.

RESULTS

Forty-one eyes of 41 patients were treated with CXL technique. The mean age was 27.97 ± 6.97 years (95% CI 25.77-30.17, range 18-44.06 years).

Compared to the preoperative data, corneal thickness was significantly thinner at 15 minutes and 30 minutes during treatment ($p < 0.001$) using Pentacam HR. The average decrease was 108.95 ± 48.6 μm at 15 minutes and 112.35 ± 47.3 μm at 30 minutes intraoperatively using Pentacam HR. The rate of the decrease showed no correlation with the initial corneal thickness values ($r = 0.16$; $p = 0.7$). Three days after the operation, no statistically significant deviation was found between the measured points and the initial values using Pentacam ($p = 0.17$) and ultrasound device ($p = 0.32$). No further changes were detected during the follow-up period. Our corneal pachymetry data are summarized in Tables I and II and illustrated in Figure 1. Intraoperative images were captured by Pentacam HR and Visante OCT (Fig. 2). A hyper-reflective corneal tissue zone can be clearly seen in Pentacam HR images but is not distinctly visible in Visante OCT.

TABLE I - CORNEAL THICKNESS MEASUREMENTS IN μm AT THE CORNEAL APEX, THE THINNEST CORNEAL POINT (MIN), AND PUPIL CENTER (PUPIL) PRIOR TO COLLAGEN CROSSLINKING, INTRAOPERATIVELY, AND IN THE POSTOPERATIVE FOLLOW-UP PERIOD, USING PENTACAM HR

	Mean	SD	95% CI	Minimum	Maximum
Preoperative					
Apex	463.61	53.454	446.513-480.703	299.33	564.67
Min	446.74	51.692	430.209-463.273	298	559.67
Pupil	472.83	49.577	456.978-488.689	321.33	569.33
15 minutes					
Apex	354.66	63.088	334.482-374.835	199.67	468.33
Min	341.99	65.61	321.009-362.975	165.67	459
Pupil	359.13	64.101	338.633-379.634	192	481.67
30 minutes					
Apex	351.25	53.277	334.212-368.289	233.67	458
Min	336.43	59.024	317.556-355.310	208.67	453
Pupil	354.16	55.581	336.383-371.934	240	464
3 days					
Apex	484.53	78.136	459.201-509.859	275.67	666.33
Min	466.56	81.392	440.171-492.940	222.67	646.33
Pupil	490.33	76.608	465.500-515.167	295.33	667.67
1 week					
Apex	467.45	62.92	447.057-487.849	298.33	592
Min	450.72	60.394	431.141-470.296	290.67	561
Pupil	476.83	59.366	457.585-496.073	322	593.67
1 month					
Apex	456.68	55.596	438.895-474.456	285.33	560
Min	441	53.345	423.939-458.060	276.67	540
Pupil	467.25	49.241	451.502-482.998	334	570
3 months					
Apex	453.78	53.843	434.369-473.194	291.33	567
Min	437.65	52.382	418.759-456.531	283.67	558.33
Pupil	467.24	49.303	449.464-485.015	327.67	573.33
6 months					
Apex	444.29	53.633	422.154-466.432	296.33	526.33
Min	429.39	51.364	408.186-450.589	293	506
Pupil	457.76	47.916	437.983-477.540	329.67	532.67
12 months					
Apex	441.02	55.775	412.343-469.696	294	523
Min	426.35	52.725	399.244-453.462	289.67	502.67
Pupil	454.59	49.264	429.258-479.917	329.33	531

CI = confidence interval; SD = standard deviation.

[Author: Please provide high res images fig. 1 & 2.]

TABLE II - CORNEAL THICKNESS MEASUREMENTS IN μM AT THE CENTER OF THE CORNEA PRIOR TO COLLAGEN CROSSLINKING, INTRAOPERATIVELY, AND IN THE POSTOPERATIVE FOLLOW-UP PERIOD, USING AN ULTRASOUND PACHYMETER

	Mean	SD	95% CI	Minimum	Maximum
Preoperative	475.80	58.763	457.007-494.593	317	590
15 minutes	339.88	57.529	321.476-358.274	205	460
30 minutes	330.03	44.748	315.714-344.336	207	460
3 days	474.71	69.928	450.693-498.735	311	580
1 week	475.80	56.937	457.591-494.009	310	565
1 month	470.49	56.764	451.560-489.413	310	567
3 months	468.74	54.216	447.294-490.188	320	557
6 months	463.36	48.423	443.372-483.348	325	555
12 months	460.12	54.690	431.999-488.237	325	554

CI = confidence interval; SD = standard deviation.

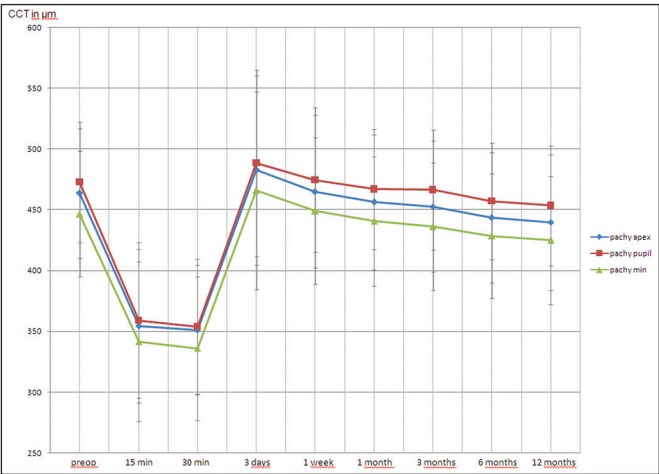


Fig. 1 - Mean corneal thickness changes at 15 minutes and 30 minutes during collagen crosslinking and 3 days, 1 week, 1, 3, 6, and 12 months after treatment. In every case, thickness measurements at the corneal apex (apex), pupil center (pupil), and the thinnest point of the cornea (min) were recorded. CCT = central corneal thickness.

DISCUSSION

The purpose of CXL treatment is to establish new chemical bonds in corneal stroma induced by riboflavin and 370-nm UVA light. On one hand, riboflavin acts as a photosensi-

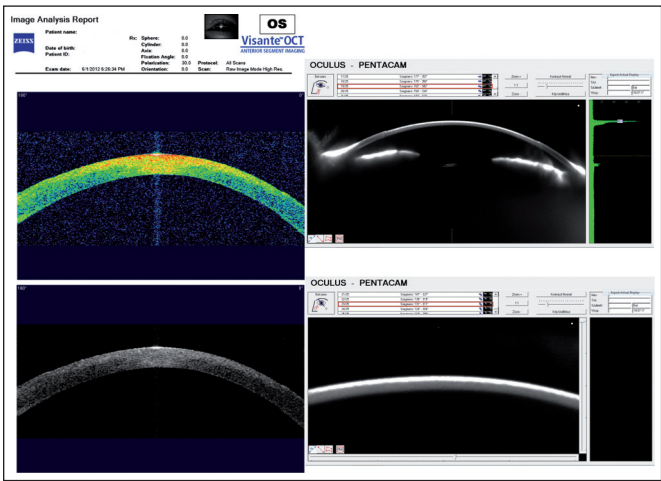


Fig. 2 - Pentacam HR and Visante optical coherence tomography (OCT) images captured at 15 minutes during collagen crosslinking treatment. Hyperreflective corneal tissue zone can be seen in Pentacam HR pictures (right) and not distinctly visible in Visante OCT pictures (left).

tizer during treatment; on the other hand, it protects the areas below the treatment zone from the effects of the UVA radiation (5, 6). The aim of the treatment is to increase the rigidity and the biomechanical strength of the cornea. This therapy has been widely used for the treatment of progressive keratoconus (1-4), although its other diagnostic abilities are also well-known (13). Both objective (14, 15) and subjective (16) improvements were reported in eyes with keratoconus after CXL treatment, even in subjects under 18 (17).

As collagen bonds are established at a depth of 250-350 μm in the anterior stroma, called the treatment zone (5, 18-21), and for the protection of the endothelium, a minimum of 400 μm stromal thickness is suggested (5, 22). However, according to other publications, a minimum of 330 μm of initial thickness is also sufficient for CXL after corneal swelling (23). Transepithelial CXL or customized pachymetric guided epithelial debridement may be beneficial in cases where corneal thickness is thinner than 400 μm (24, 25). Our study comprised 2 patients who had refused any surgical procedure, where the initial corneal thickness value was under 300 μm . According to our experience, it is safe to use isotonic riboflavin after corneal swelling is achieved with hypotonic riboflavin solution. However, in our practice a preoperative treatment limit of at least 400 μm is used.

Our study aimed to reveal the process of how the well-known changes in corneal thickness after CXL therapy

(7, 10, 26, 27) occur at a less investigated intraoperative stage and in the follow-up period.

Administering isotonic riboflavin, Greenstein et al (28) measured pupil, apical, and the thinnest corneal thickness as being 23-24.6 μm thinner 1 month after treatment using Scheimpflug analysis. At 1 year, apex and pupil center thicknesses returned to the baseline; however, the thinnest pachymetry remained slightly lower than baseline and similar values were found for pupil, apex, and thinnest pachymetry thicknesses (28). The gradually increasing values did not reach preoperative readings until 1 year after the operation. Data were compared with ultrasound pachymetry, but were analyzed only preoperatively (28). A number of other studies reported changes in corneal thickness after CXL treatment and, in every case, a decrease was observed (7-10, 26, 27). Despite the findings mentioned above, corneal thickening was reported 3 days after CXL in rabbit cornea, which ceased only 1 week later (29). So it seems that corneal tissues may react differently to the treatment due to structural differences.

It has also been suggested that changes in corneal thickness observed using Scheimpflug analysis can be an artefact (30, 31) resulting from postoperative corneal haze (8, 9, 32). In our study, ultrasonic measurements were also carried out subsequent to treatments, which showed similar results preoperatively and from 1 week after CXL. However, intraoperatively and at the 3-day visit, ultrasound pachymetry measured smaller CCT than Pentacam. The possibly altered refractive index of crosslinked corneas can be the background of this difference.

Results obtained with Scheimpflug analysis and ultrasound measurements correlate well with each other. The thinning of the cornea and stromal haze can be explained by structural and physiologic wound-healing changes developing after treatment (33), though the mechanism of corneal re-thickening remains unexplained.

A limitation of our study is that subsequent to de-epithelialization no Pentacam HR images were captured. Normal epithelial thickness was reported as $53.4 \pm 4.6 \mu\text{m}$ (34), a value supported by the data reported by Kymionis et al of $43 \mu\text{m}$ (35). When this mean epithelial thickness is subtracted from the thickness that we measured preoperatively, the decrease in the corneal thickness remains highly significant ($p < 0.01$).

During pachymetric measurements, corneal thickness was analyzed at the thinnest point and in the pupil center. The readings of Henriquez et al (36) showed a mean

corneal thinning from $56 \mu\text{m}$ to $13 \mu\text{m}$ at the thinnest point of the cornea 1 and 12 months after treatment. Vinciguerra et al (14) found a significantly thinner cornea in the pupil center even 1 year after treatment; however, they showed no significant change vs the thinnest pachymetry location in eyes with keratoconus 1 year after the CXL operation. Both authors performed central CXL operation. In our study, the corneal thickness returned to the preoperative level on postoperative day 3, which remained stable in each of the 3 measured points even at the end of the 1-year follow-up.

Publications suggest corneal thinning in the early postoperative stage, so UVA light can reach corneal endothelium intraoperatively. Therefore, hypoosmolar riboflavin solution is suggested in order to achieve corneal iatrogenic swelling (37), although according to previous research it has only a short-term effect and does not even last until the end of the treatment (38).

Publications found in the literature clearly describe the CCT decrease observed after CXL treatment, though only a few and contradicting reports were carried out on the characteristics of CCT decrease in the intraoperative phase (35, 38, 39). Using isotonic solution, Kymionis et al (35) detected a mean $75 \mu\text{m}$ CCT decrease between epithelial removal and riboflavin instillation with ultrasound pachymetry measurements; however, they found no further changes in corneal thickness during irradiation. These data correlate well with our data regarding the decrease in thickness. Kaya et al (38) measured corneal thickness at 5 different times: the thinnest pachymetry data decreased to a mean of $376 \mu\text{m}$ after the epithelial removal and by a mean of $55 \mu\text{m}$ after the 30-minute application of the isotonic riboflavin solution. The 10-minute application of hypotonic riboflavin resulted in the cornea thickening by a mean of $59 \mu\text{m}$. Ten and 30 minutes after the instillation of isotonic riboflavin solution, the thinnest pachymetric readings showed a decrease with a value of 50 and $65 \mu\text{m}$. Holopainen and Krootila (39) measured corneal thickness both preoperatively and postoperatively using ultrasound pachymeter and found a thinning of a mean $87 \mu\text{m}$ during the 1-hour operation. Depending on the initial corneal thickness, they administered isotonic or hypotonic solution. Compared to the preoperative CCT, they reported a significantly lower value 1 month after the operation and found the same value 6 months after treatment. In our study, corneal thickness reached the initial value 3 days after treatment.

In summary, a significant decrease in corneal thickness can be observed during CXL treatment applied for treating keratoconus. However, this change is temporary and the value measured initially is reached 3 days after treatment. Further investigations are suggested in order to determine whether this change may have any consequences or whether it is of any significance.

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Address for correspondence:
Gabor Nemeth, MD, PhD
Department of Ophthalmology
University of Debrecen
Nagyerdei Blvd. 98
H-4012 Debrecen
Hungary
nemeth222@yahoo.com

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