

# Can We Measure Corneal Biomechanical Changes After Collagen Cross-Linking in Eyes With Keratoconus?—A Pilot Study

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**Purpose:** To assess changes in biomechanical properties of human cornea after treatment of keratoconus with UV-A–riboflavin corneal collagen cross-linking (CXL).

**Design:** Single-center, prospective, interventional study.

**Methods:** Ten eyes of 10 patients aged  $26.5 \pm 5.7$  (mean  $\pm$  SD) years with progressive keratoconus were treated with UV-A–riboflavin CXL and assessed with the Ocular Response Analyzer (ORA) that measured corneal hysteresis (CH), corneal resistance factor (CRF), Goldmann-correlated intraocular pressure (IOPg), and corneal compensated intraocular pressure (IOPcc). Intraocular pressure was also measured by Goldmann applanation tonometry (GAT-IOP). Patients were assessed with ORA preoperatively, at week 1, months 1, 3, and 6 after treatment. Postoperative measurements at each visit were compared with preoperative values.

**Results:** CH and CRF were transiently elevated after cross-linking treatment, with the difference not statistically significant ( $P > 0.3$ ). IOPcc and IOPg were statistically significantly higher at 1 week and 1 month but not subsequently ( $P < 0.04$ ). GAT-IOP was statistically significantly higher at 1 week and at 1 and 3 months ( $P < 0.01$ ).

**Conclusions:** There were no significant differences in corneal biomechanical properties, as measured with the ORA parameters CH and CRF, after CXL in keratoconus. IOPcc, IOPg, and GAT-IOP values were transiently elevated after CXL treatment in our study. Whether this reflects a measurement artifact resulting from corneal changes or true elevation of intraocular pressure is unclear.

**Key Words:** cross-linking, keratoconus, collagen, riboflavin–UV-A, Ocular Response Analyzer

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Keratoconus is a progressive ectasia of the cornea resulting from noninflammatory thinning of the corneal stroma.<sup>1</sup> Visual impairment from myopia and irregular astigmatism is commonly noted in adolescence and progresses thereafter.<sup>2</sup> The initial management of keratoconus is based on refractive correction with spectacles and contact lenses. Further ectatic progression ultimately necessitates corneal transplantation in 10%–20% of patients.<sup>3</sup>

Corneal collagen cross-linking (CXL) using UV-A and riboflavin was proposed recently as treatment to halt the progression of keratoconus.<sup>4</sup> Others have proposed that similar cross-linking of collagen fibers occurs naturally in diabetic corneas and thus provides protection against the progression of keratoconus in patients with diabetes.<sup>5,6</sup> Previous *in vitro* studies have shown increased corneal rigidity<sup>7–9</sup> and increased corneal resistance to enzymatic degradation<sup>10</sup> after CXL. *In vivo* evaluation of changes in biomechanical properties after human CXL treatment has not been described. The Ocular Response Analyzer (ORA; Reichert, Inc, Buffalo, NY) can be used to assess *in vivo* corneal biomechanical properties, presented by 2 parameters, corneal hysteresis (CH) and corneal resistance factor (CRF). It also provides noncontact measurement of intraocular pressure (IOP) through the parameters Goldmann-correlated IOP (IOPg) and corneal compensated IOP (IOPcc). Detailed description of this instrument has been previously published.<sup>11</sup> Briefly, the instrument measures corneal response to indentation by a rapid air pulse using an electro-optical system. The air puff causes the cornea to move inward, passing a defined point of applanation and into a slight concavity. After reaching the pressure peak, the pressure of the air pulse decreases and the cornea returns to its normal configuration, passing again the defined point of applanation. The electro-optical system monitors this entire process and calculates the above parameters. CH represents the absolute difference between the 2 pressure values causing force-in (P1) and force-out (P2) applanations and provides a measure of viscous damping of the cornea. The CRF is derived from the formula  $(P1 - kP2)$ , where  $k$  is a constant. The constant  $k$  was determined from an empirical analysis of the relationship between both P1 and P2 and central corneal thickness (CCT) to develop a parameter more strongly associated with CCT than CH.<sup>12</sup> IOPg is the average of the 2 IOP measurements at the applanation points. IOPcc is a pressure measurement that uses the information provided by CH to provide an IOP that is less affected by CCT or corneal curvature.<sup>11</sup>

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The aim of our study was to prospectively assess in vivo the changes in biomechanical properties of human corneas with the ORA after treatment of keratoconus with UV-A–riboflavin CXL.

## METHODS

Patients with keratoconus were prospectively recruited from the Cornea Outpatient Clinic of the Assaf Harofeh Medical Center. Inclusion criteria were progressive keratoconus documented clinically within the past 12 months by astigmatic refraction and/or topography, age over 18 years, no previous ocular surgery, no corneal opacities, minimal corneal thickness of 400  $\mu\text{m}$ , and no wearing of contact lenses for 1 month before initial evaluation and treatment. Patients were treated with UV-A–riboflavin CXL under aseptic conditions using topical preoperative anesthesia with oxybuprocaine hydrochloride 0.4% drops (Localin; Fisher Pharmaceutical Labs). Treatment included 7-mm-diameter corneal deepithelization, instillation of 0.1% riboflavin in 20% dextran solution (Peschke Meditrade GmbH, Huenenberg, Switzerland) every 5 minutes for 40 minutes, and corneal irradiation with UV-A 3  $\text{mW}/\text{cm}^2$  (UV-X; Peschke Meditrade GmbH) for 30 minutes, 5 cm from the cornea. After the procedure, patients were treated with topical antibiotic (Oflox, ofloxacin 0.3%; Allergan) 4 times a day for 7 days, topical corticosteroid (Sterodex; dexamethasone 0.1%, Fisher Pharmaceutical Labs) 4 times a day for 1 month, and the eye was dressed with a soft therapeutic contact lens (Ocular Sciences, Ltd, Southampton, United Kingdom) for 3 days.

Patients were assessed before and at week 1, months 1, 3, and 6 after treatment. Each examination included measurement of best-corrected visual acuity, corneal topography, IOP by Goldmann applanation tonometry (GAT-IOP), slit-lamp and fundus examinations, and corneal biomechanical assessment using the ORA. We compared postoperative measurements at each visit with preoperative measurements.

For measurement with the ORA, each patient was seated and asked to fixate at a target light, and the measurement was taken by pressing a button on a personal computer linked to the ORA. A noncontact probe scans the central corneal area and releases an air puff. Measured IOP, CH, and CRF are displayed on the monitor. For each patient, we obtained 3 readings of good quality, defined as having a waveform with 2 distinct peaks, and recorded the average for each parameter.

This study was approved by the Institutional Ethics Committee of Assaf Harofeh Medical Center, and a written informed consent was obtained from each subject after the nature and intent of the study had been fully explained. The study protocol was consistent with the tenets of the Declaration of Helsinki.

## Statistical Analysis

The data are presented as frequency or mean  $\pm$  standard deviation. Paired 2-tailed Student *t* test was used to assess differences between the compared groups in CH, CRF, CCT, IOPcc, IOPg, and GAT-IOP. The distributions of values within each data set were evaluated graphically. A *P* value of 0.05 was

selected for the threshold of statistical significance. Analyses were performed using Excel (Microsoft, Corp, Redmond, WA).

## RESULTS

Ten eyes of 10 patients were treated with UV-A–riboflavin CXL and assessed prospectively with the ORA. There were 3 females and 7 males with mean age  $26.5 \pm 5.7$  years (range 18–37 years). Mean maximum keratometry ( $K_{\text{max}}$ ) reading was  $53.07 \pm 6.3$  diopters, and mean CCT was  $470 \pm 36.6 \mu\text{m}$ .

Table 1 presents the mean CH and CRF values before treatment and week 1, months 1, 3, and 6 after treatment. Mean CH and CRF values were transiently increased (at 1 week and 1 month) after CXL, with the differences from baseline not statistically significant. Figures 1 and 2 present the maximal, minimal, and median values with interquartile ranges of CH and CRF before and after CXL treatment.

Mean IOPcc and IOPg were statistically significantly higher at 1 week and 1 month after CXL compared with preoperative values (Table 2, Fig. 3). Statistically significant differences between preoperative and postoperative values were found for GAT-IOP at 1 week, 1 month, and 3 months (Table 2).

## DISCUSSION

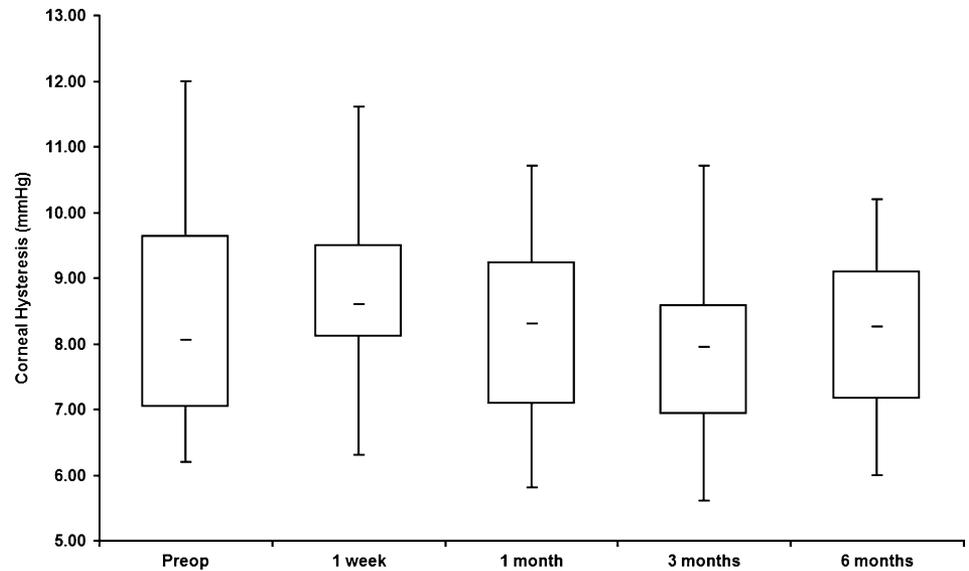
Because cross-linking of collagen supposedly halts progression of keratoconus by increasing the stiffness of the cornea,<sup>4</sup> we expected to observe changes in CH and CRF after treatment. Theoretically, therapeutically inducing cross-linking of collagen might act similarly to processes that retard keratoconus progression during aging and prolonged uncontrolled hyperglycemia.<sup>5,6,13</sup>

In this pilot short-term study, we did not observe significant changes in biomechanical properties of the cornea after CXL for keratoconus as measured in vivo by ORA. It is noteworthy that the baseline values of CH and CRF in our study were similar to those reported in previous studies of keratoconic eyes.<sup>14,15</sup>

The effect of this treatment has been previously assessed only using clinical parameters such as corneal topography and subjective refraction,<sup>4,16</sup> whereas we sought to demonstrate physical corneal changes. Previous in vitro studies described physical changes in the cornea after cross-linking. Wollensak et al<sup>8</sup> used stress–strain measurements to evaluate the effect of riboflavin–UV-A CXL on corneal rigidity in human and

**TABLE 1.** Biomechanical Properties Before and After CXL Treatment

Time	Mean $\pm$ SD (mm Hg)	
	CH	CRF
Before	8.44 $\pm$ 1.82	7.15 $\pm$ 1.77
1 wk	8.62 $\pm$ 1.56 ( <i>P</i> = 0.1)	8.48 $\pm$ 1.55 ( <i>P</i> = 0.82)
1 mo	8.22 $\pm$ 1.50 ( <i>P</i> = 0.77)	7.91 $\pm$ 1.54 ( <i>P</i> = 0.32)
3 mo	7.88 $\pm$ 1.57 ( <i>P</i> = 0.46)	7.1 $\pm$ 1.51 ( <i>P</i> = 0.94)
6 mo	8.14 $\pm$ 1.32 ( <i>P</i> = 0.32)	7.16 $\pm$ 1.45 ( <i>P</i> = 0.87)



**FIGURE 1.** Box and whisker plots (smallest, median, and largest values with interquartile range) showing CH before treatment (preoperative) and on 1 week, 1 month, 3 months, and 6 months thereafter.

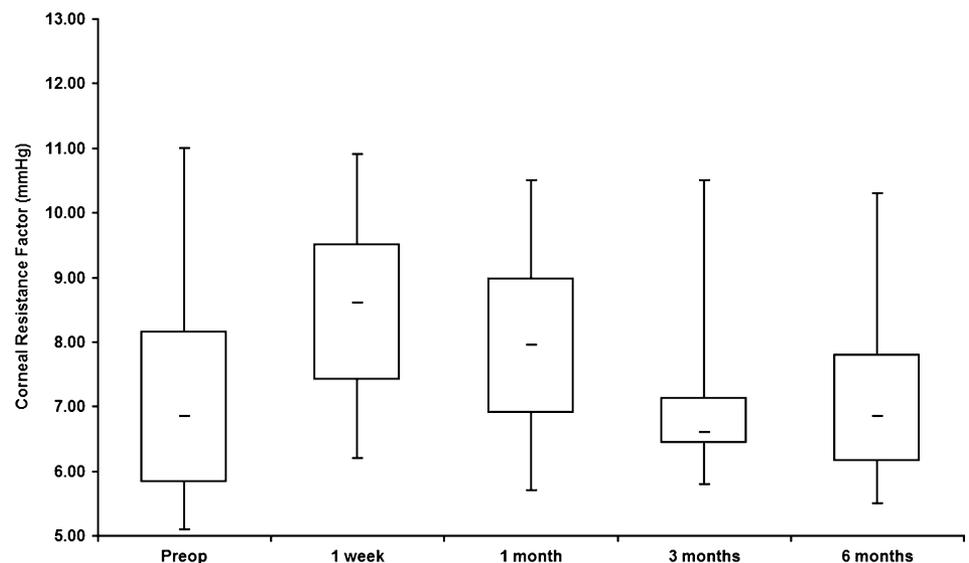
porcine corneas. Using microcomputer-controlled biomaterial tester, they showed significant increase in rigidity in both human and porcine corneas. Dupps et al<sup>9</sup> used an ultrasonic device to evaluate the effect of human and porcine corneal cross-linking with glutaraldehyde. Through measuring sonic wave propagation time between 2 transducers positioned on the corneal surface, they found increased corneal stiffening after glutaraldehyde CXL. Noguera et al described an in vitro model of porcine cornea evaluation with ORA after CXL.<sup>17</sup> In their study, significant elevation in both CH and CRF was seen in the subgroup treated with riboflavin-UV-A.

Our results, namely, the lack of biomechanical corneal changes after CXL, are in disagreement with the in vitro changes mentioned above. This may be explained, in part, by the different methodologies used to assess these changes and by some inherent differences between in vivo and in vitro

models. Additionally, it is plausible that biomechanical changes did occur but were too subtle to be measured by ORA.

One possible weakness of our study is the relatively short follow-up time. However, it has been shown in vivo<sup>15,16</sup> that after riboflavin-UV-A CXL treatment, stromal keratocyte repopulation was complete by 6 months and was accompanied by disappearance of stromal edema. Therefore, we think that it is unlikely that a greater amount of change would be observed with longer follow-up. Another weakness is the small number of participants. Because our pilot study included a small number of eyes, we cannot rule out that statistically significant changes could be demonstrated with a larger sample size. Further study is needed in this regard.

Recently, more advanced analysis of the raw data provided by ORA has been suggested in addition to the CH and CRF parameters in biomechanical evaluation. This



**FIGURE 2.** Box and whisker plots (smallest, median, and largest values with interquartile range) showing CRF before treatment (preoperative) and on 1 week, 1 month, 3 months, and 6 months thereafter.

**TABLE 2.** Intraocular Pressure Before and After CXL Treatment

Time	Mean $\pm$ SD (mm Hg)		
	IOPcc	IOPg	GAT-IOP
Before	13.6 $\pm$ 2.06	10.2 $\pm$ 1.63	10.1 $\pm$ 1.66
1 wk	16.7 $\pm$ 2.40 ( $P = 0.01$ )	14.1 $\pm$ 2.21 ( $P < 0.001$ )	14.2 $\pm$ 2.73 ( $P < 0.001$ )
1 mo	16.5 $\pm$ 3.57 ( $P = 0.04$ )	13.2 $\pm$ 3.55 ( $P = 0.03$ )	13.5 $\pm$ 3.12 ( $P = 0.01$ )
3 mo	15.3 $\pm$ 3.48 ( $P = 0.19$ )	11.6 $\pm$ 3.17 ( $P = 0.23$ )	12.0 $\pm$ 1.25 ( $P = 0.01$ )
6 mo	14.7 $\pm$ 2.87 ( $P = 0.21$ )	11.2 $\pm$ 2.89 ( $P = 0.33$ )	12.2 $\pm$ 1.14 ( $P = 0.16$ )

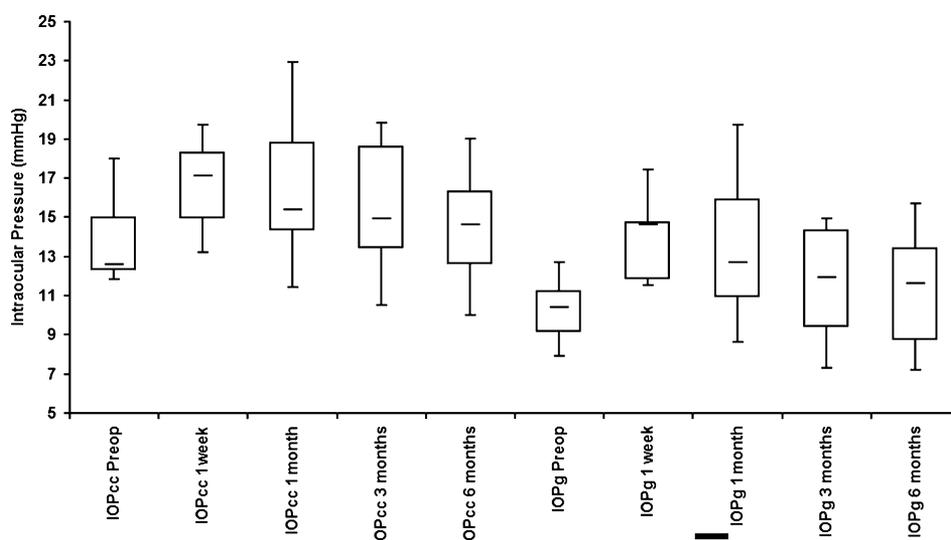
analysis is performed using graphically presented waves and includes comparison of signal peak amplitudes and shape,<sup>19</sup> width of infrared peaks at their mid-height point, and slope of air pulse during the 2 applanation events.<sup>20</sup> Once the accuracy and reliability of these analytic tools are established, they can potentially be used to reexamine the effect of CXL.

Various factors might influence the measurement of postoperative IOP including corneal biomechanical properties, corneal thickness, and corneal curvature.<sup>21–24</sup> The ORA permits in vivo noncontact evaluation of pressure metrics through IOPcc and IOPg. Noncontact IOP evaluation by ORA was examined in previous studies.<sup>23,25,26</sup> Martinez-de-la-Casa et al<sup>26</sup> and Broman et al<sup>23</sup> showed that IOP values in glaucomatous eyes as measured by the ORA were higher, on average, than values measured with Goldmann applanation tonometer. The relationship between ocular characteristics and interaction of different types of tonometers with the eye is complex, although IOPcc seems to provide estimation of IOP that is less influenced by corneal properties than Goldmann tonometer.<sup>11,25</sup>

In our study, IOP measurements were taken by ORA and Goldmann tonometer on every follow-up visit. GAT-IOP values up to 3 months postoperatively were statistically significantly higher compared with preoperative values. A statistically significant elevation of noncontact IOP measurements (IOPcc and IOPg) was detected 1 week and 1 month after treatment. Whether these changes reflect true elevation

of IOP is uncertain. Previously, Wollensak et al<sup>4</sup> found no significant change in IOP after CXL measured with the Goldmann tonometer. In the above mentioned study by Dupps et al,<sup>9</sup> it was reported that IOP measurements taken with TonoPen in vitro, after CXL with glutaraldehyde, were higher despite keeping constant IOP. They presumed that stiffening of the human cornea is responsible for such artifactual increase in measured IOP. The Siena Study Group,<sup>16,18</sup> by using confocal microscopy, described transient stromal edema appearing shortly after riboflavin–UV-A CXL and disappearing by 3 months. They also reported increased CCT measured by ultrasonic pachymetry after CXL but without significant IOP elevation as measured by TonoPen. Thus, early postoperative IOP changes in our study may be a measurement artifact following changes in ocular characteristics, like increased corneal thickness, and not a real IOP elevation. Because the increased IOP was observed as early as 1 week after CXL, we think it unlikely that it is secondary to response to topical steroid treatment, but this cannot be ruled out.

In conclusion, in the present study, we did not observe significant change in corneal biomechanical properties, as measured with the ORA parameters CH and CRF, after CXL in keratoconus. Measured IOP values were transiently elevated after CXL treatment in our study. Whether this reflects a measurement artifact resulting from corneal changes or true elevation of IOP is unclear.



**FIGURE 3.** Box and whisker plots (smallest, median and largest values with interquartile range) showing preoperative IOP (IOPcc preoperative and IOPg preoperative) and corresponding values of both IOPcc and IOPg on 1 week, 1 month, 3 months, and 6 months after cross-linking treatment.

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