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Original research

Assessment of the changes in corneal biomechanical properties after collagen cross-linking in patients with keratoconus

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Abstract

Purpose: To assess the changes in biomechanical properties of the cornea after treatment of keratoconus patients with UV-A/riboflavin corneal collagen cross-linking (CXL) using Corvis ST (Oculus, Wetzlar, Germany) and Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Inc., Buffalo, NY, USA) devices.

Methods: In this prospective, observational case series, 48 eyes from 48 consecutive patients with progressive keratoconus were enrolled. Patients with history or signs of ocular disorders other than keratoconus, previous eye surgery, systemic diseases, or inability to cooperate with any measurement device were excluded. Corvis ST and ORA images were obtained at baseline and 4 months after CXL. The primary outcome measures comprised Corvis ST corneal biomechanical factors [time of highest concavity (T), time of applanation 1 (T1), time of applanation 2 (T2), length of applanation 1 (L1), length of applanation 2 (L2), velocity of applanation 1 (V1), velocity of applanation 2 (V2), deformation amplitude (DA), peak distance (PD), and radius (R)] and the ORA parameters [corneal hysteresis (CH), corneal resistance factor (CRF), Goldmann-related IOP (IOPg), cornea-compensated IOP (IOPcc), and waveform score (WS)].

Results: The mean [± standard deviation (SD)] age of patients was 20 ± 5 years, and 27 (56%) were male. At baseline, the averages of the refraction, mean keratometry, and keratometric astigmatism were −3.0 ± 1.8 diopter (D), 47.0 ± 1.8 D, and 3.5 ± 1.5 D, respectively. According to Corvis ST, L2 increased from 0.83 ± 0.25 mm at baseline to 1.15 ± 0.57 mm after CXL; and V2 decreased from −0.81 ± 0.08 to −0.94 ± 0.26 m/s ($P = 0.001$ and $P = 0.032$, respectively). ORA parameters showed significant decrease in the CRF (from 7.82 ± 1.72 to 7.21 ± 1.05 mmHg; $P = 0.036$) and increase in the WS (from 4.58 ± 2.55 to 6.12 ± 1.92; $P = 0.002$).

Conclusions: According to in vivo observation with Corvis ST and ORA, CXL induces significant changes in corneal biomechanical properties in cases with keratoconus. The parameters with significant changes (L2 and V2) may reflect increased stiffness of the treated cornea. The importance of such observations should be elucidated in future studies.

Keywords: Corneal biomechanics; Corneal collagen cross-linking; Corvis ST; Ocular response analyzer

Introduction

Keratoconus is a non-inflammatory cone-like ectasia of the cornea, which is usually bilateral and progresses over time.[1] Despite extensive research in this field, the exact etiopathology of keratoconus is not fully understood so far. It has been suggested that increased levels of lysosomal and proteolytic

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enzymes, a decreased concentration of protease inhibitors, reduced number of collagen cross-links, and pepsin digestion higher than normal may induce an overall structural weakness of the corneal tissue in cases with keratoconus.[2–6] Decreased mechanical corneal stability plays a crucial role in the progressive protrusion of the keratoconic cornea,[7] resulting in significant impairment of visual acuity owing to irregular astigmatism, progressive myopia, corneal thinning, and central corneal scarring.[1]

Corneal collagen cross-linking (CXL) with UV-A and riboflavin has been used increasingly in the management of keratoconus to stabilize the cornea and stop the progression of the condition.[8] The CXL induces changes in the physicochemical properties of the collagen and has a stiffening effect on it that stabilizes the corneal stroma and increases its resistance to enzymatic degradation.[9–14]

Different instruments have been developed for the direct measurement of the biomechanical properties of the cornea.[15,16] The Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Inc., Buffalo, NY, USA) and Corvis ST (Oculus, Wetzlar, Germany) are two commercially available instruments that evaluate the biomechanical response of the cornea to an air puff-induced deformation. The ORA uses a precisely metered, progressively escalating jet of air that leads to the indentation of the cornea as determined by an electro-optical collimation detector system. Once the desired indentation is achieved, the air jet pressure decreases, allowing the cornea to resume its original shape. Because there is a time-lag to decrease pressure of the air jet, the cornea actually indents mildly beyond the intended applanation point. This allows the detection of a second applanation point as the cornea returns from its over-applanated state to its normal convex shape. The first applanation pressure point (P1) and the second applanation pressure point (P2) are used to derive four output parameters: Goldmann correlated IOP (IOPg); corneal compensated IOP (IOPcc); corneal hysteresis (CH); and corneal resistance factor (CRF). Corvis ST is a non-contact tonometer that measures intraocular pressure (IOP) and corneal thickness and also provides additional information about biomechanical responses of the cornea using dynamic Scheimpflug imaging analysis (4330 frames per second) during an air-puff corneal deformation.[17]

Several previous studies have evaluated corneal biomechanical changes after CXL with controversial outcomes. Using adequate sample size, robust methodology, and incorporating both the ORA and Corvis ST devices, the present study aimed to prospectively assess the changes in biomechanical properties of cornea after CXL in keratoconus patients using Corvis ST and ORA devices.

Methods

Study population

In this prospective, observational case series, ORA, and Corvis ST corneal biomechanical parameters were recorded at baseline and 4 months after CXL for 48 eyes from 48 consecutive eligible patients with keratoconus. The rationale behind selecting ORA and Corvis ST for the purpose of the present study was the availability of these devices. For patients with bilateral procedure, only the eye that had undergone CXL first (usually with more severe keratoconus) was included. A comprehensive eye examination including Snellen visual acuity measurement, slit-lamp biomicroscopy, and fundus examination using a 90-diopter non-contact lens was performed on each eye. Patients with history or signs of eye disorders (e.g., uveitis, glaucoma, corneal scars or opacities, Fuchs's dystrophy, and diabetic retinopathy), previous eye surgery, systemic diseases, or inability to cooperate with any measurement device were excluded. The study protocol adhered to the tenets of the Declaration of Helsinki, and detailed informed consent was signed by all participants. This study was approved by the Ethics Committee at Shiraz University of Medical Sciences.

Procedure

All CXL procedures were performed by the same surgeon (R.S.) using accelerated technique as previously described in detail.[18] In brief, the eye was prepared by instilling a topical anesthetic and povidone-iodine drops. Then the central epithelium was removed using 20% alcohol and a surgical spear followed by copious irrigation of the eye with normal saline. The CXL was done by UV-A KXL system (Avedro Inc., Waltham, MA) using riboflavin 0.1%. Device settings comprised the wavelength (continuous wave) of 365 ± 10 nm with energy dose of 5.4 J/cm^2 and average intensity of 9 mV/cm^2. The illumination diameter was 7.5 mm and 9.5 mm for the small and medium settings, respectively. The working distance was set at 45 mm, and the soak/interval and the treatment times were both 15 minutes. The same setting was used for all patients. At the end, the treated eye received levofloxacin 0.5% and homatropine 2% eye drops, and a therapeutic contact lens was applied onto the cornea.

Measurements

An autorefractometer (Canon R-50; Canon Inc., Tokyo, Japan) and Pentacam HR (Oculus Optikgeräte GmbH, Wetzlar, Germany) were used to measure refractions and keratometry measurements, respectively.

IOP, central corneal thickness (CCT), and ocular biomechanical parameters were obtained using Corvis ST. This device measures and records biomechanical response of the cornea to an air-puff at the moment of the first applanation, second applanation, and highest concavity events. Corvis ST corneal biomechanical parameters comprise time to reach applanation [time of applanation 1 (T1), time of applanation 2 (T2)], the length of the applanated cornea [length of applanation 1 (L1), length of applanation 2 (L2)], and corneal movement velocity during applanation [velocity of applanation 1 (V1), velocity of applanation 2 (V2)] at the moment of first and second applanations, respectively. It also records the time [time of highest concavity (T)], deformation amplitude

(DA), distance between bending points of the cornea [peak distance (PD)], and concave radius of curvature (R) at the point of highest concavity.

From the ORA printouts, CH, CRF, IOPg, IOPcc, and waveform score (WS) were recorded. CH is calculated as the difference in air pressures between the first and second applanations: (P1 − P2). CRF is measured using the formula (P1 − kP2), where k is a constant that was developed through empirical evaluation of the relationship between P1, P2, and CCT. The value of k is more strongly associated with CCT than CH; hence, CRF is relatively independent of IOP. The IOPg is calculated as the average of the 2 applanation pressures that are captured during inward and outward movements of the cornea. IOPcc is derived from IOPg based on the corneal biomechanical data.

Each device was calibrated at the beginning of the study, and then at recommended intervals as per manufacturer recommendations. All measurements were performed by qualified operators following instructions provided by the manufacturers of devices.

Statistical analysis

Data were recorded and analyzed using IBM SPSS Statistics software version 21 (SPSS Inc., Chicago, IL) and MedCalc version 12.2.1 (MedCalc Software, Mariakerke, Belgium). Descriptive statistical results were presented as mean ± standard deviation (SD). Normality of variables was evaluated using Shapiro-Wilk test. Paired sample T test or Wilcoxon Signed Rank test, whenever appropriate, were used to compare pre- and post-CXL measurement. Linear regression analysis was used to explore possible associations between baseline characteristics and changes in corneal biomechanical parameters. A P value of less than 0.05 was deemed statistically significant.

Results

The mean (±SD) age of patients was 20 ± 5 years (range, 10–37 years), and 27 (56%) were male. At baseline, the averages of the refraction, mean keratometry, and keratometric astigmatism were −3.0 ± 1.8 diopter (D), 47.0 ± 1.8 D, and 3.5 ± 1.5 D, respectively. The baseline and post-CXL measurements of Corvis ST and ORA parameters are presented in Tables 1 and 2. From the Corvis ST parameters, L2 (mean difference, 0.32 mm; 95% CI, 0.15 to 0.50) and V2 (mean difference, −1.30 m/s; 95% CI, −0.20 to −0.06) showed significant changes after CXL (Fig. 1); L2 increased after CXL for about 38.5%, and V2 showed increase in velocity for about 16% (the minus sign of the V2 represents the inward to outward direction of corneal movement). CRF (mean difference, −0.65 mmHg; 95% CI, −1.25 to −0.05) and WS (mean difference, 1.54; 95% CI, 0.64 to 2.43) were the only ORA parameters that showed statistically significant changes after CXL (Fig. 2); CRF decreased for approximately 8.8% after CXL, and the WS improved from 4.58 ± 2.55 to 6.12 ± 1.92.

Table 1
Comparison of Corvis ST parameters between measurements obtained before and after corneal collagen cross-linking.

	Baseline	After CXL	P value
IOP, mmHg	15.2 ± 2.1	15.0 ± 2.0	0.702
CCT, μm	473 ± 29	475 ± 27	0.688
T1, milliseconds	7.08 ± 0.28	7.05 ± 0.31	0.538
L1, mm	1.80 ± 0.25	1.77 ± 0.20	0.439
V1, m/s	0.12 ± 0.02	0.12 ± 0.03	0.512
T2, milliseconds	19.87 ± 0.20	19.85 ± 0.26	0.367
L2, mm	0.83 ± 0.25	1.15 ± 0.57	0.001
V2, m/s	−0.81 ± 0.08	−0.94 ± 0.26	0.032
T, milliseconds	17.05 ± 0.57	17.01 ± 0.52	0.394
DA, mm	1.16 ± 0.12	1.12 ± 0.09	0.062
PD, mm	4.54 ± 0.94	4.34 ± 1.10	0.348
R, mm	5.69 ± 0.64	5.54 ± 0.50	0.165

CXL: Corneal collagen crosslinking; IOP: Intraocular pressure; CCT: Central corneal thickness; T1: Time of applanation 1; L1: Length of applanation 1; V1: Velocity of applanation 1; T2: Time of applanation 2; L2: Length of applanation 2; V2: Velocity of applanation 2; T: Time of highest concavity; DA: Deformation amplitude; PD: Peak distance; R: Radius.

Table 2
Comparison of Ocular Response Analyzer (ORA) parameters between measurements obtained before and after corneal collagen cross-linking (CXL).

	Baseline	After CXL	P value
IOPg, mmHg	12.4 ± 2.7	11.5 ± 2.6	0.062
IOPcc, mmHg	15.1 ± 2.4	14.5 ± 3.0	0.297
CH, mmHg	8.57 ± 1.58	8.37 ± 1.52	0.596
CRF, mmHg	7.82 ± 1.72	7.21 ± 1.05	0.036
WS	4.58 ± 2.55	6.12 ± 1.92	0.002

CXL: Corneal collagen crosslinking; IOP: Intraocular pressure; IOPg: Goldmann-related IOP; IOPcc: Cornea-compensated IOP; CH: Corneal hysteresis; CRF: Corneal resistance factor; WS: Waveform score.

A multiple step-wise linear regression analysis model was applied to explore the possible association between the age, sex, spherical equivalent refraction, and Pentacam keratometric and pachymetric indices, and the found changes in corneal biomechanical parameters showed that for the post-CXL changes in L2, the baseline L2 was the only independent determinant with negative association, such that the smaller baseline L2 showed greater change toward larger values after CXL ($P = 0.015$). For the changes in V2, baseline L2 and IOPcc had independent association ($P = 0.008$ and $P = 0.004$, respectively), in such a way that the greater baseline L2 or IOPcc were associated with greater increase in the velocity of the second applanation. Within ORA parameters, baseline WS showed independent and negative association with post-CXL changes in WS ($P < 0.001$), which means the smaller baseline WS was associated with greater increase in the post-CXL WS. In addition, baseline CRF was the only significant factor that showed independent association with changes in CRF after CXL ($P < 0.001$). Accordingly, the greater baseline CRF was associated with greater decrease in post-CXL CRF. Post-CXL changes in WS was not correlated with changes in the CRF (Pearson correlation coefficient, 0.002; $P = 0.991$).

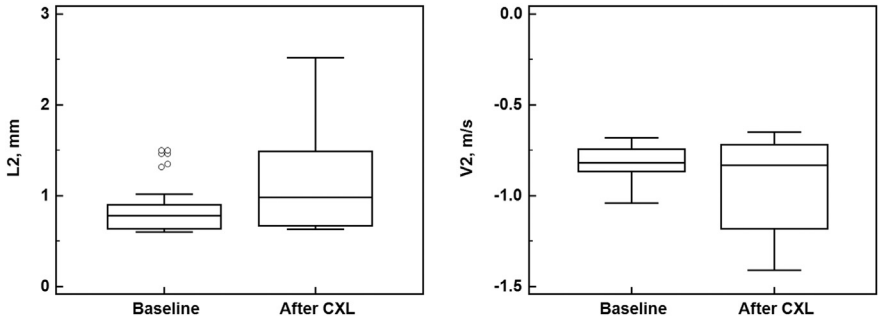

Fig. 1. Box and Whisker plot for length of applanation 2 (L2) and velocity of applanation 2 (V2) parameters of the Corvis ST at baseline vs. after corneal collagen cross-linking (CXL).

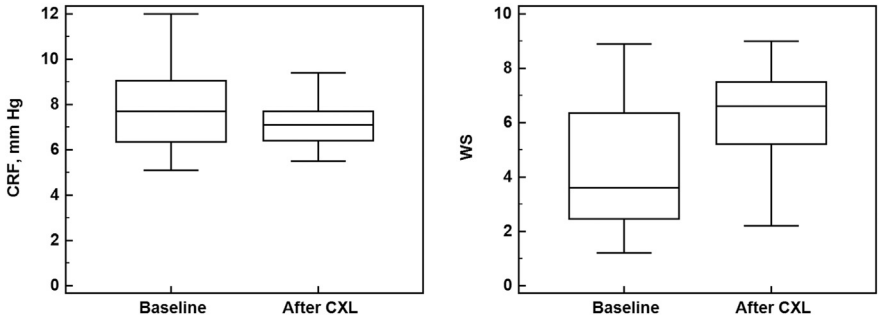

Fig. 2. Box and Whisker plot for corneal resistance factor (CRF) and waveform score (WS) parameters of the Ocular Response Analyzer (ORA) at baseline vs. after corneal collagen cross-linking (CXL).

Discussion

The results of our study demonstrate significant changes in some corneal biomechanical parameters after treatment of keratoconus patients with CXL as determined by Corvis ST and ORA devices. However, several parameters remained unchanged. One of the limitations of the ORA and Corvis ST devices is that the surface they applanate is too large to capture subtle changes in corneal biomechanics. This could justify, at least in part, why most parameters remained unchanged after CXL. In addition, both devices do not consider the fluid pressure and volume in the anterior chamber, and hence the measurement obtained by these instruments might not properly reflect the biomechanical properties of the cornea. Furthermore, the measurements with the ORA could be interfered by light scatter from surface corneal irregularities (such as keratoconus) because the ORA uses infrared specular reflection beam to measure applanation pressure. Altogether, these issues might raise concern about the accuracy and validity of the ORA and Corvis ST in measuring corneal biomechanical properties, which could be elucidated in future studies.

Several experimental and clinical studies indicated that CXL causes a long-term increase in corneal biomechanical rigidity that results in corneal stabilization and improvement of vision. Basic laboratory studies have demonstrated that CXL by stiffening the human cornea by more than 300% caused a long-term increase in corneal biomechanical rigidity with a primary treatment effect in the anterior 300 microns of the corneal stroma.[10,12,19–21] In addition; CXL induces the formation of high molecular-weight collagen polymers, with increased chemical stability.[22] It has also been demonstrated that CXL increase the collagen fiber diameter in the anterior corneal stroma by 12.2%.[23] Confocal and thermomechanical studies of the cornea after CXL have supported these structural findings.[24,25] On the other hand, clinical studies with long-term follow-up demonstrated effectiveness of CXL for keratoconus as documented by improvement of uncorrected and best spectacle-corrected visual acuity and pachymetric and keratometric indices as well as a decrease in total corneal wavefront aberrations.[26–28]

Considering ORA, CRF and WS were the only ORA parameters that showed statistically significant change after CXL in our study. Several previous studies have measured corneal biomechanical changes after CXL in a series of keratoconus patients using ORA and did not find a statistically significant difference between preoperative and post-CXL values.[29–33] In contradiction to those studies and in agreement with ours, De Bernardo et al.[34] found a significant decrease in CRF (from 6.6 to 6.2; $P = 0.01$) but not in CH at 3 months after CXL in keratoconus patients. Salman[35] also reported trends toward decreased CRF at the follow-up exam of 6 months or more after CXL for pediatric keratoconus. Therefore, the non-significant report on ORA biomechanical parameters after CXL in aforementioned studies might be a result of insufficient sample size or device repeatability issues due to inferior waveform quality (i.e., WS) in keratoconus eyes[36,37] (as also demonstrated in our study), and should be interpreted with

caution. Future well-designed studies with larger sample size or meta-analysis of the available data in the current literature might help to clarify the issue. In addition, we do not consider the apparent decrease in the CRF after CXL synonymous to the decrease in the biomechanical strength of the cornea. The interpretation of ORA biomechanical parameters might be different for the same cornea before vs. after treatment compared to corneas of different eyes. This issue should also be elucidated in future research.

Regarding the Corvis ST, there are fewer studies on biomechanical changes after CXL in keratoconus. Bak-Nielsen et al. examined 9 eyes of 8 patients before and after CXL.[38] Patients were measured with Corvis at baseline and on the mean day 97 after CXL (range, 41−103 days). In their study of 9 eyes, measurements before and after CXL showed a significant difference only in highest concavity (HC) DA, HC time, and T2. When the authors considered their findings with regard to the direction of change, only HC time followed their expectation as it increased after CXL. HC, DA, and T2 increased after CXL; however, the changes were opposite to the values in normal corneas. The authors concluded that standard parameters of the Corvis ST cannot readily be used for diagnosis of keratoconus or to document the effect of CXL in follow-up examinations.[38] Another study has compared the outcomes of accelerated CXL vs. conventional CXL.[39] The authors have demonstrated that there were no significant differences in the postoperative changes of corneal biomechanical responses from ORA or Corvis ST between the procedures.[39] Although the primary aim of this study performed by Tomita et al. was to compare the outcomes of accelerated versus conventional CXL, detailed scrutinizing of their results showed that from Corvis ST printouts, they only compared DA, distance between corneal bending points, and R. Comparison of pre-treatment with post-treatment values of these parameters showed that statistically significant change has occurred only in distance between corneal bending points in accelerated CXL group, and none of these parameters was changed significantly in conventional CXL group.[39] In our study, we only found post-CXL change in the L2 and the V2, implicating that the parameters related to the second applanation may be more sensitive to detect post-CXL biomechanical changes in the cornea. We hypothesize that an increase in L2 and modulus of the V2 might reflect extra-stiffness induced by the treatment. Intuitively, these changes could be explained by increased corneal stiffness. Fuchsluger et al. found the same for the V2, but the opposite for the L2.[16] However, the validity and importance of such findings are unclear and could become the subject of future research.

In conclusion, CXL seems to induce measurable corneal biomechanical changes in cases with keratoconus, which could be detected by ORA or Corvis ST. The importance of such alterations is not clear at present and demands further research. Studies with larger sample size or meta-analysis of the data from available literature may help to overcome inherent limitations such repeatability issue of the devices to offer more reliable data.

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