Corneal Biomechanics After Accelerated Cross-linking: Comparison Between 18 and 9 mW/cm² Protocols

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ABSTRACT

PURPOSE: To determine 1-year corneal biomechanical changes after accelerated corneal cross-linking in patients with progressive keratoconus and compare them between 5-minute (18 mW/cm²) and 10-minute (9 mW/cm²) protocols.

METHODS: In this non-randomized clinical trial, cases in both groups were examined with the Corneal Visualization Scheimpflug Technology (Corvis ST; Oculus Optikgeräte GmbH, Wetzlar, Germany) at baseline and at 6 and 12 months after treatment. Extracted indices included intraocular pressure (IOP), central corneal thickness (CCT), first and second applanation times, lengths, and velocities (T1, T2, L1, L2, V1, and V2), highest concavity time (HCT), deformation amplitude (DA), peak distance between bending points, and radius of curvature.

RESULTS: Mean patient age, baseline maximum keratometry, CCT, and IOP were similar between groups. After adjusting for CCT and baseline values with repeated measures analysis of covariance, at 1 year after the procedure, IOP (13.14 ± 1.41 vs 12.12 ± 1.49 mm Hg, $P = .034$) and T1 (6.84 ± 0.20 vs 6.67 ± 0.23 ms, $P = .036$) were higher in the 5-minute group, but T2 (21.31 ± 0.27 vs 21.58 ± 0.28 ms, $P = .007$), HCT (16.06 ± 0.51 vs 16.31 ± 0.48 ms, $P = .017$), and DA (1.03 ± 0.09 vs 1.10 ± 0.08 mm, $P = .028$) were lower. Other inter-group differences were not statistically significant (all $P > .050$). All 1-year changes were independent of cone position (all $P > .050$).

CONCLUSIONS: At 1 year after cross-linking in cases of mild and moderate keratoconus, corneal biomechanics appeared stable or stronger than baseline with both 5- and 10-minute protocols. However, mild cases who had the 5-minute protocol showed better improvement based on Corvis ST indices.

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Corneal cross-linking (CXL) with riboflavin is known to halt the progression of ectasia by creating additional bonds in the corneal stroma and strengthening it.[1] The safety and efficacy of standard CXL (3 mW/cm² for 30 minutes) have been shown in long-term studies.[2,3] Because both clinicians and patients favor shorter procedure times, accelerated protocols were suggested based on the Bunsen–Roscoe law of reciprocity. By applying this law to CXL, irradiation time can be reduced by increasing the intensity of the energy. Schumacher et al.'s[4] experiments with porcine corneal strips indicated that treatment with the 9-minute CXL protocol (10 mW/cm²) increased Young's modulus similar to the standard protocol. Results presented by Mazzotta et al.[5] at the 3rd Joint International Congress in Italy in 2013 indicated similar morphological changes with the 4-minute CXL protocol (30 mW/cm²) compared to their 10-minute approach (12 mW/cm²).

Corneal biomechanical properties can be measured with the Ocular Response Analyzer (ORA; Reichert Technologies, Depew, NY) and the Corneal Visualization Scheimpflug Technology (Corvis ST; Oculus Optikgeräte GmbH, Germany). In this study, we aimed to compare changes in corneal biomechanics after two accelerated CXL protocols (18 mW/cm² for 5 minutes and 9 mW/cm² for 10 minutes) using the Corvis ST.

PATIENTS AND METHODS

In this study, patients with progressive keratoconus undergoing 5-minute (18 mW/cm² for 5 minutes) or 10-minute

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(9 mW/cm^2 for 10 minutes) accelerated CXL were recruited from a non-randomized clinical trial under way at Noor Eye Hospital in Tehran since June 2013. Inclusion criteria were a diagnosis of progressive keratoconus (at least 1.00 diopter [D] increase in maximum keratometry [Kmax], manifest cylinder, or manifest refraction spherical equivalent in the past 12 months), age between 15 and 35 years, Kmax less than 55.00 D, and minimum corneal thickness of 400 µm. Patients with a history of any ocular surgery or disease were excluded. In both groups, hard and soft contact lens users were instructed not to wear them for 3 weeks and 3 days, respectively, before the procedure and examinations. Cases were subgrouped by the position of the cone: central keratoconus if the cone was in the central 3 mm of the cornea and inferior keratoconus if beyond the central 3 mm.

The Ethics Committee of Tehran University of Medical Sciences approved the study. The study adhered to the tenets of the Declaration of Helsinki at all stages. A written informed consent was obtained from each participant.

Surgical Technique

For both groups, local anesthesia was obtained using proparacaine hydrochloride 0.5%, three times at 5-minute intervals, after which the epithelium of the central 9 mm was scraped off and the eyelid speculum was removed. Then 0.1% riboflavin in 20% dextran (StreuliPharma, Uznach, Switzerland) was instilled onto the corneal surface every 3 minutes for 30 minutes. For irradiation, the CCL 365 (PESCHKE Meditrade GmbH, Waldshut-Tiengen, Germany) with an intensity of 18 mW/cm^2 was used in the 5-minute group and the LightLink-CXL (LightMed, San Clemente, CA) with an intensity of 9 mW/cm^2 was used in the 10-minute group. After rinsing the corneal surface, a soft bandage contact lens (Night & Day; Ciba Vision, Duluth, MN) was placed and one drop of levofloxacin was instilled. The post-CXL prescription included levofloxacin and betamethasone 0.1% eye drops four times daily and preservative-free artificial tears as needed. After reepithelialization was observed in daily follow-up examinations, the bandage contact lens was removed, levofloxacin was discontinued, and betamethasone was continued four times daily for another week.

Examinations Before and After CXL

Corvis ST indices were measured at baseline and at 6 and 12 months after CXL. All Corvis ST imaging was performed by the same optometrist before and after CXL. Extracted indices included intraocular pressure (IOP), corrected IOP (IOP correction based on the Dresden correction table), central corneal thickness (CCT), first and second applanation times (T1 and T2), first and second applanation lengths (L1 and L2), first and second applanation velocities (V1 and V2), highest concavity time (HCT), and corneal characteristics at the HCT including deformation amplitude (DA), peak distance between the bending points (PD), and radius of curvature (RC-HCT).

Statistical Analyses

One-year changes between the two groups were compared using repeated measures analysis of covariance (ANCOVA). In the analysis, the correlation between fellow eyes of bilateral cases and baseline CCT were adjusted for. The normality of data distribution was evaluated using the one-sample Kolmogorov–Smirnov test. All indices had a normal distribution except PD ($P < .001$), for which we applied the Mann–Whitney test for inter-group comparisons. In comparing changes between the two groups, given their limited sample sizes, we used power analysis to assess borderline P values.

RESULTS

The study sample included a total of 55 eyes: 30 in the 5-minute group and 25 in the 10-minute group. Of these, 26 (86.7%) in the 5-minute group and 24 (96.0%) in the 10-minute group completed the 1-year follow-up. The mean age of these cases was 23.3 ± 5.7 years (range: 16 to 34 years) in the 5-minute group and 22.4 ± 6.0 years (range: 15 to 31 years) in the 10-minute group ($P = .58$). Also, 63.2% of the 5-minute group and 52.6% of the 10-minute group ($P = .511$) were men. **Table 1** summarizes visual acuity and topographic indices for each group at baseline and 1 year after the procedure. Of the studied indices, baseline CCT was significantly different between the two groups ($P = .003$), but not other parameters (all $P > .05$). Therefore, CCT was adjusted for as a covariate in the analysis.

Parameters measured by the Corvis ST at baseline and follow-up visits are presented in **Table 2**.

For covariate analyses, we adjusted for baseline CCT and the baseline value of each parameter being tested. Results showed that at 1 year after CXL, IOP ($P = .034$) and T1 ($P = .036$) were higher in the 5-minute group, whereas T2 ($P = .007$), RC-HCT ($P = .017$), and DA ($P = .015$) were lower in the 5-minute group. Intergroup differences in other indices were not statistically significant (all $P > .050$). All of the above analyses were repeated after entering cone position as a covariate. Cone position had no significant relationship with the 1-year change of any of the studied indices (all $P > .050$).

One-year changes were significantly different between the 5- and 10-minute groups for IOP ($P = .024$), T1 ($P =$

TABLE 1

Comparison of Baseline and 1-Year Visual Acuity and Topographic Indices (as Measured With the OPD Scan III) Between the 5-Minute and 10-Minute Groups

	Baseline		1 Year Postoperatively		
Parameter	**5-Minute**	**10-Minute**	**5-Minute**	**10-Minute**	***P*[a]**
UDVA (logMAR)	0.41 ± 0.46	0.48 ± 0.36	0.39 ± 0.41	0.40 ± 0.31	.485
CDVA (logMAR)	0.14 ± 0.17	0.17 ± 0.16	0.14 ± 0.17	0.11 ± 0.12	.506
Kmax (D)	46.60 ± 2.20	47.6 ± 2.10	46.10 ± 2.60	47.50 ± 2.60	.162
Kmin (D)	43.70 ± 1.50	43.70 ± 1.90	43.70 ± 1.00	43.50 ± 1.30	.929
Irregularity	1.81 ± 1.04	1.88 ± 1.21	1.77 ± 0.97	1.87 ± 1.13	.818

UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity; Kmax = maximum keratometry; D = diopters; Kmin = minimum keratometry
[a]Inter-group comparison of baseline values.
The OPD Scan III is manufactured by Nidek, Fremont, CA.

TABLE 2

Corvis ST–Measured Parameters in the 5-Minute and 10-Minute Cross-linking Groups

	5-Minute Group				10-Minute Group			
Parameter	**Baseline**	**6 Months**	**12 Months**	**1-Year Change**	**Baseline**	**6 Months**	**12 Months**	**1-Year Change**
DII								
IOP (mm Hg)[a]	12.73 ± 1.80	13.34 ± 1.61	13.14 ± 1.41	+0.41 ± 1.39	12.57 ± 2.18	12.70 ± 2.02	12.12 ± 1.49	-0.33 ± 1.85
Corrected IOP (mm Hg)	14.86 ± 1.63	15.79 ± 1.64	15.29 ± 1.19	+0.52 ± 1.33	15.92 ± 1.77	16.08 ± 1.82	15.43 ± 1.56	-0.36 ± 1.88
CCT (μm)	498.8 ± 30.5	492.8 ± 30.2	498.2 ± 31.9	-0.6 ± 14.7	466.2 ± 22.3	465.5 ± 25.4	467.3 ± 23.8	+0.8 ± 10.9
First applanation								
T1 (ms)[a]	6.81 ± 0.31	6.91 ± 0.25	6.84 ± 0.20	+0.04 ± 0.21	6.76 ± 0.33	6.76 ± 0.29	6.67 ± 0.23	-0.07 ± 0.31
L1 (mm)	1.71 ± 0.16	1.76 ± 0.04	1.75 ± 0.06	+0.04 ± 0.17	1.66 ± 0.14	1.72 ± 0.13	1.69 ± 0.10	+0.03 ± 0.14
V1 (ms)	0.13 ± 0.03	0.13 ± 0.02	0.13 ± 0.02	+0.00 ± 0.02	0.14 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	-0.01 ± 0.03
Highest concavity								
HCT (ms)[a]	16.27 ± 0.57	16.22 ± 0.66	16.06 ± 0.51	-0.31 ± 0.56	16.16 ± 0.40	16.32 ± 0.59	16.31 ± 0.48	+0.20 ± 0.56
DA (mm)[a]	1.04 ± 0.12	1.01 ± 0.08	1.03 ± 0.09	-0.01 ± 0.07	1.07 ± 0.11	1.09 ± 0.11	1.10 ± 0.08	+0.02 ± 0.08
PD (mm)	4.21 ± 1.12	3.83 ± 1.29	4.34 ± 1.07	+0.12 ± 1.37	4.43 ± 1.00	4.48 ± 0.97	3.98 ± 1.22	-0.44 ± 1.29
RC-HCT (mm)	6.08 ± 0.99	6.23 ± 0.83	6.33 ± 1.14	+0.30 ± 0.75	5.60 ± 0.61	5.92 ± 0.70	5.99 ± 0.68	+0.41 ± 0.76
Second applanation								
T2 (ms)[a]	21.41 ± 0.33	21.29 ± 0.32	21.31 ± 0.27	-0.13 ± 0.24	21.56 ± 0.39	21.57 ± 0.45	21.58 ± 0.28	-0.01 ± 0.36
L2 (mm)	1.66 ± 0.41	1.58 ± 0.34	1.65 ± 0.34	-0.02 ± 0.49	1.50 ± 0.40	1.66 ± 0.31	1.58 ± 0.39	+0.10 ± 0.39
V2 (ms)	-0.37 ± 0.06	-0.39 ± 0.05	-0.39 ± 0.04	-0.01 ± 0.05	-0.42 ± 0.06	-0.43 ± 0.07	-0.42 ± 0.06	+0.00 ± 0.06

DII = deformation independent index; IOP = intraocular pressure; CCT = central corneal thickness; T1, T2, L1, L2, V1, V2 = first and second applanation times, lengths, and velocities; HCT = highest concavity time; DA = deformation amplitude; PD = peak distance between the bending points; RC = radius of curvature
[a]1-year changes and adjusted values at 1 year were significantly different between the two groups.
The Corvis ST is manufactured by Oculus Optikgeräte, Wetzlar, Germany.

.020), T2 ($P = .031$), RC-HCT ($P = .009$), and DA ($P = .020$), but not for other indices (all $P > .050$) (**Table 2**).

In the 5-minute group, increased IOP correlated inversely with baseline Kmax ($P = .046$) and directly with irregularity ($P = .024$). Also, increased T1 ($P = .047$) and decreased T2 ($P = .025$) showed a direct significant correlation with baseline irregularity. Changes in RC-HCT and DA were not significantly correlated with vision and topographic variables.

In the 10-minute group, only increased T1 showed a direct significant correlation with baseline CCT. Other corneal biomechanical indices had no significant relationship with Kmax, irregularity, CDVA, or CCT (all $P > .050$).

Baseline measurements were not possible for 4 eyes in the 5-minute group and one eye in the 10-minute group due to severe corneal irregularity. Changes at 6 and 12 months after CXL in these cases are shown in

Table A (available in the online version of this article). Trends after CXL in these cases were similar to the mean changes observed in the main groups.

DISCUSSION

Corneal biomechanics provide some of the most important indices in the assessment of changes in keratoconus and their clinical follow-up. In keratoconus, changes in the collagen structure lead to corneal weakening.[6] The Corvis ST is a device for the measurement of corneal biomechanical properties. The repeatability, reproducibility, and inter-index correlation of its measurements have been reported previously, and certain indicators (CCT, DA, T1, IOP, and RC-HCT) have shown better repeatability.[7-9] Because thinner corneas such as keratoconic corneas are weak and less rigid, their response to deformation should be faster and their recovery should be slower.[10] Indicators measured by the Corvis ST have shown significant differences between normal and keratoconic eyes.[9] After CXL, these parameters are expected to change in line with corneal stiffening. Bak-Nielsen et al.[9] reported significant changes in DA, HCT, and T2 indices after CXL.

Because CCT affects corneal biomechanical properties,[11] we adjusted for its effect in the inter-group comparison analysis in this study so that inter-group differences in biomechanical changes can be judged with better certainty. Lack of significant difference in corrected IOP compared to the significant change in IOP in our study pointed to the effect of CCT on Corvis ST indices. Among measured Corvis ST indices, changes in T1, T2, DA, and HCT significantly differed between the 5- and 10-minute CXL groups.

Adjusted analysis showed that at 1 year after treatment, T1 was higher in the 5-minute group than the 10-minute group and T2 was the reverse. DA was also lower in the 5-minute group. Given that the difference of corneal curvature was not significant between the two groups, these observations suggest that the corneas in the 5-minute group were more resistant to flattening, their response to the air puff was slower, and they had a faster rebound. In other words, the corneas in the 5-minute group became stiffer than those in the 10-minute group. A lower DA in the 5-minute group can be the cause for the lower HCT in this group. Therefore, the direction of post-CXL change of HCT should be examined and interpreted in relation to the changes in other Corvis ST indices. Even V1 and V2 indicated slower deformation and faster rebound in the 5-minute group. However, given the limited sample size, the study lacks sufficient power to show any significant difference between the two groups.

Tomita et al.[12] compared the accelerated 3-minute method with the standard protocol and, based on the indices of DA, PD, and RC-HCT, suggested that the two approaches had comparable strengthening effects on corneal biomechanics. Of note, contrary to the standard group, DA increased in the accelerated group, although the inter-group difference in this regard was not statistically significant. Perhaps the difference between our study and theirs is controlling for confounders such as baseline CCT and the correlation between fellow eyes in bilateral cases.

A point worth mentioning in the current study is that although baseline Kmax was not significantly different between the two groups, the 1.00 D difference might be indicative of faster disease progression in the 10-minute group and better biomechanical strengthening of the cornea in the 5-minute group. It would be necessary to do a randomized study with a longer follow-up time to investigate this subject. It should also be noted that the two different ultraviolet irradiation systems we used in this study are similar in terms of ultraviolet lightwave length, spot size, beam power, and voltage, and although Mencucci et al.[13] suggested that their differences in light emission are negligible, a potential role cannot be definitely overruled.

Overall, it could be concluded that at 1-year after CXL in cases of mild and moderate keratoconus with a minimum corneal thickness of 400 µm, both the 5- and 10-minute protocols strengthened or maintained corneal biomechanics. However, in early keratoconus, the 5-minute method showed better improvement based on Corvis ST indices.

Wernli et al.[14] demonstrated a greater increase in corneal stiffness with an intensity level of 20 mW/cm^2 compared to 10 mW/cm^2; the intergroup difference was not statistically significant using non-parametric tests, which have a lower power compared to parametric tests (ANCOVA). In vitro studies[15,16] indicate that higher ultraviolet intensities in CXL support better surgical efficacy in the corneal anterior and mid stoma, which contribute most to increased corneal stiffness; however, the depth of CXL is shallower. There is not yet consensus on the best CXL depth for the human cornea. Studying porcine corneas, Hammer et al.[17] reported comparable corneal stiffness with 5- and 10-minute protocols. In human studies, 5- and 30-minute protocols appeared to have similar results in terms of vision, refraction, corneal thickness, endothelial cell count, and corneal biomechanics; the standard protocol only provided better flattening.[18,19] These inconsistencies and the possibility of reduced cross-linked mass at higher intensities further highlight the need for long-term comparative studies of corneal biomechanical changes.

AUTHOR CONTRIBUTIONS

Study concept and design (HH, SA); data collection (HH, MM); analysis and interpretation of data (SA, SM, RG, AF); writing the manuscript (SA); critical revision of the manuscript (HH, SM, MM, RG, AF); statistical expertise (SA); administrative, technical, or material support (MM, AF); supervision (HH)

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TABLE A

Corvis ST–Measured Parameters in the 5-Minute and 10-Minute Cross-linking Groups Whose Baseline Measurements Were Not Possible Due to Severe Corneal Irregularity

	5-Minute (4 Eyes)		10-Minute (1 Eye)	
Parameter	**6 Months**	**12 Months**	**6 Months**	**12 Months**
Deformation independent index				
IOP (mm Hg)	12.67 ± 1.89	12.33 ± 3.55	10.00	11.00
Corrected IOP (mm Hg)	15.00 ± 1.28	14.53 ± 2.46	15.00	15.50
CCT (μm)	492.0 ± 35.2	495.3 ± 33.9	425.0	438.0
First applanation				
T1 (ms)	6.73 ± 0.31	6.72 ± 0.46	6.36	6.51
L1 (mm)	1.68 ± 0.12	1.76 ± 0.02	1.65	1.59
V1 (ms)	0.14 ± 0.02	0.14 ± 0.03	0.12	0.11
Highest concavity				
HCT (ms)	16.40 ± 0.23	16.25 ± 0.27	16.40	16.40
DA (mm)	1.05 ± 0.13	1.08 ± 0.19	1.03	0.98
PD (mm)	4.88 ± 0.13	4.96 ± 0.38	2.64	2.15
Radius (mm)	6.10 ± 1.50	6.43 ± 1.27	6.68	6.26
Second applanation				
T2 (ms)	21.45 ± 0.31	21.57 ± 0.68	21.62	21.46
L2 (mm)	1.58 ± 0.47	1.64 ± 0.20	1.29	1.76
V2 (ms)	-0.47 ± 0.01	-0.45 ± 0.20	-0.35	-0.29

IOP = intraocular pressure; CCT = central corneal thickness; T1, T2, L1, L2, V1, V2 = first and second applanation times, lengths, and velocities; HCT = highest concavity time; DA = deformation amplitude; PD = peak distance between the bending points
The Corvis ST is manufactured by Oculus Optikgeräte, Wetzlar, Germany.