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Assessment of accelerated cross-linking with different energy protocols: A comparative study

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Abstract--Purpose: To evaluate the safety and the efficacy of accelerated corneal cross-linking with two different energy protocols. Design Prospective, randomized, interventional study. Patients and Methods: 48 eyes of 26 patients aged 18-38 years with progressive keratoconus. Group A underwent accelerated cross-linking (24 eyes, 5.4 J/cm²), Group B treated with higher ultraviolet-A irradiance (24 eyes, 7.2 J/cm²). Changes in corrected distance visual acuity (CDVA), keratometric data, densitometric data, and specular microscopy were recorded preoperatively and at the 1st, 3rd, and 6th month postoperatively. One month postoperatively, the demarcation line was measured using anterior segment optical coherence tomography. Results: A mean corneal demarcation line was 231.43±27.18 μm in group A and 282.3±21.4 μm in group B and was significantly deeper in group B (P<0.05). Corneal densitometry in the anterior corneal layers was peaked at the 3rd month follow up in both groups. In contrast, middle and posterior stromal layers peaked at the 6th month in both groups (P<0.05). There were no statistically significant changes in CDVA, keratometric values, and endothelial cells in the two groups. Conclusion: We found that accelerated and modified accelerated cross-linking protocols were effective and safe in halting keratoconus progression after a follow-up period of 6 months.

Keywords--corneal collagen cross-linking, corneal densitometry, corneal demarcation line, Keratoconus

Introduction

Keratoconus is a progressive corneal degenerative disorder that causes corneal thinning and irregular astigmatism. In the past two decades, various keratoconus treatment modalities, such as corneal collagen cross-linking (CXL), have advanced significantly. Cross-linking is the only treatment that halts keratoconus progression. It exhibited effective and safe results in stabilizing progressive keratoconus. The standard cross-linking entails exposure of the cornea to 3 mW/cm² ultraviolet-A (UVA) light for 30 minutes, resulting in cumulative energy of 5.4 J/cm² after instillation of riboflavin. (1) Based on the reciprocity rule, the same photochemical effect as the standard protocol can be achieved by reducing the irradiation time and increasing the intensity (Bunsen-Roscoe law). (2) There has been a lot of interest in developing novel CXL protocols to minimize UVA exposure time, patient and doctor discomfort, and potential side effects. In terms of corneal biomechanical response and clinical outcomes, accelerated cross-linking has been found to be comparable to standard cross-linking. (3) It was suggested that the photochemical reciprocity law could not explicitly apply to cross-linking in living corneal tissue. Thus, increasing the total delivered energy can create an efficient treatment for keratoconic eyes comparable to the already effective standard cross-linking protocol. (4) Although cross-linking has become an important intervention to stop the progression of ectatic corneal disorders, a decrease in visual acuity is common during the early postoperative period. (5) This reduction in the visual acuity might be caused by backward light scatter from the cornea due to loss of stromal transparency. The main cause for corneal haze after cross-linking seems to be a complicated corneal healing process involving keratocytes transformation into myofibroblasts, resulting in stromal remodeling. (6) The current study aims to assess the safety and efficacy of accelerated cross-linking using two different energy protocols in progressive keratoconus through studying corneal topographic changes, corneal stromal demarcation line depth, the safety on the corneal endothelium, and corneal stromal clarity.

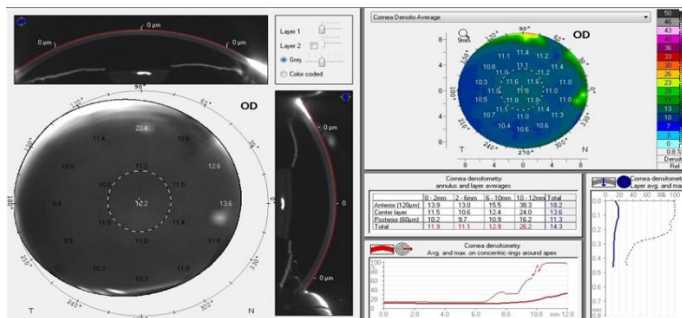
Subjects and Methods

This is a prospective, randomized, comparative, interventional study enrolling patients with progressive keratoconus. The current research was reviewed and approved by the ethical committee of Kasralainy faculty of medicine, Cairo University, and followed the tenets of the Declaration of Helsinki. All patients provided informed consent and ensured their ability to follow the study instructions. All cases were done in the period between April 2018 to April 2020. Progression of keratoconus was defined by one or more of the following parameters: an increase of 1.00 diopter (D) or more in the maximum keratometry value (Kmax), an increase of 0.50 D or more in manifest refraction spherical equivalent (MRSE), or an increase of 1.00 D or more in the manifest cylinder in two successive visits over six months period. Exclusion criteria were corneal pachymetry of <400 μm at the thinnest location, severe eye dryness and central corneal opacity, history of herpetic corneal infection, concomitant corneal infections, severe allergic conjunctivitis, or concurrent autoimmune diseases.

The study included 48 eyes from 26 patients, seven of which were removed due to missed follow-up, leaving 41 eyes from 22 patients. The 41 eyes were allocated into two groups: group A and group B. In group A, 21 eyes were subjected to accelerated cross-linking (A-CXL) with the power of 18 mW/cm² for 5 minutes with a total energy of 5.4 J/cm². In group B, 20 eyes were treated with A-CXL with the intensity of 18 mW/cm² for 6 minutes and 40 seconds with a total energy of 7.2 J/cm². Randomization of the two groups was done by a person other than the surgeon using a computer program (Random Allocation Software version 1.0.0) to ensure concealment.

Preoperative assessment

Preoperative refraction, uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), specular microscopy, and Scheimpflug imaging (Pentacam, Oculus Optikgerate GmbH, Wetzlar, Germany) were obtained. Corneal densitometry is a software in the Oculus pentacam device. The examination protocol entails capturing a series of 25 images (1003 × 520 pixels) of the cornea in different meridians within 2 seconds using a homogenous blue light source. The output is expressed in the arbitrary units (grayscale units (GSU)). The GSU scale is ranged between 0 (i.e., maximum transparency with minimum light scatter) and 100 (i.e., minimum transparency with maximum light scatter). The corneal densitometry is measured over a circle with a 12 mm diameter central on the corneal apex. The 12- mm diameter area is divided into four concentric radial zones (0-2, 2-6, 6-10, 10-12 mm, and total). The output can also be divided based on the corneal depth into anterior: anterior 120 μm, posterior: the most posterior 60 μm of the cornea, and central: lies between the anterior and posterior layers (Figure 1). (7)



A

haze was done using the corneal densitometry software in Oculus pentacam device. Anterior Segment-OCT examination using OCT RTVue (Optovue, Inc., Fremont, CA) was done at the 1st month follow-up visit to assess the corneal stromal demarcation line (DL) depth. Specular microscopy (TOPCON SP-1P) was done at the 1st and 6th month follow-up visits. All clinical signs were documented, and any operative and postoperative complications were recorded.

Statistical analysis

Data were statistically described in terms of mean \pm standard deviation (\pm SD), median and range, or frequencies (number of cases) and percentages when appropriate. Numerical data were tested for the normal assumption using Kolmogorov Smirnov test. Comparison of numerical variables between the study groups was done using Student t test for independent samples. Within group comparison of numerical variables was done using paired t test. Two-sided p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows. Power and sample size for the study is calculated using a statistical program according to the formula: (based on a published study) (125)

$$\text{Sample size} = 2(SD)^2 (Z\alpha/2 + Z\beta)^2 d^2$$

$$Z\alpha/2 = 1.96 \text{ at type 1 error of 5\%}$$

$$Z\beta = 0.842 \text{ (From Z table) at 80\% power}$$

$$SD = \text{Standard deviation of variable taken from previous study} = 1.80$$

$$d = \text{effect size} = \text{different between mean values}$$

$$\text{Sample size} = 2(1.80)^2 (1.96+0.842)^2 = 11 \text{ eyes in each group.} \\ (12.32-14.47)^2$$

Results

The current study involved 48 eyes of 26 patients, of which seven eyes of 4 patients have been excluded (missed follow-up), leaving 41 eyes of 22 patients (Figure 2). Of the 22 patients, 63.6% were females (14 patients), and 36.4% were males (8 patients). As shown in table 1, the mean age was 24.9 ± 4.56 years (range 18 to 38 years). The patients attended the follow-up appointments at the 1st, 3rd, and 6th months postoperatively. No postoperative complications were recorded.

Table 1
Preoperative data

Parameter	5.4 J/cm ² group	ACXL	7.2 J/cm ² group	ACXL	P-value*
Eyes	21		20		
Mean (Decimal)	CDVA	0.59 \pm 0.17		0.61 \pm 0.16	0.852*
Mean SE		-3.12 \pm 1.26		-3.08 \pm 1.08	0.905*
Mean K ₁		45.519 \pm 2.948		45.285 \pm 3.771	0.826*
Mean K ₂		49.129 \pm 2.95		49.820 \pm 4.083	0.536*

Mean K_{\max}	54.700 ±3.922	55.300 ±6.639	0.725*
Mean total corneal densitometry	13.24 ±1.903	13.54 ±1.407	0.574*
Mean endothelial cell count	2911.80 ± 120.15	2977.85 ± 151.91	0.130*

ACXL: Accelerated corneal cross-linking; CDVA: corrected distance visual acuity; SE: spherical equivalent; K_1 : flat keratometry; K_2 : steep keratometry; K_{\max} : maximum keratometry

* Intergroup differences (unpaired *t*-test)

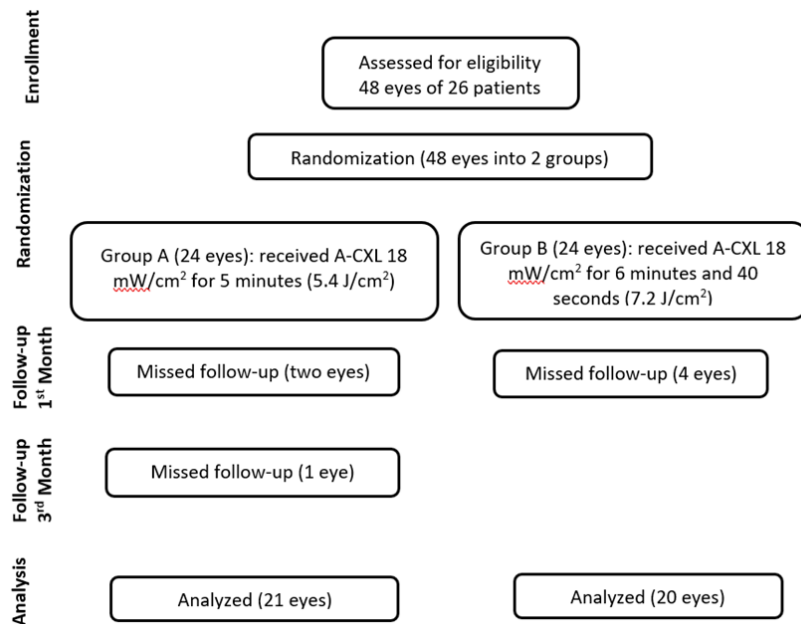


Fig. 2. Participants flow chart

Corneal stromal demarcation line depth

The mean DL depth was 231.43±27.18 μm (range 168 to 274 μm) in the first group (5.4 J/cm²) and 282.3±21.4 μm (range 243 to 314 μm) in the second group (7.2 J/cm²) (Figure 3). We found that the difference in mean DL depth between the two groups was statistically significant ($P < 0.05$, *t*-test for unpaired data). Figure 4 shows that the corneal stromal DL in patient number 7 in the second group (7.2 J/cm²) was measured at 259 μm .

Visual acuity and refraction measurements

We found that the mean CDVA was decreased significantly at the 3rd month ($P < 0.05$) in both groups, however, the difference between baseline and in 6th months follow-up values was not significant ($P > 0.05$). The changes in the mean CDVA between the two groups at the 3rd and 6th months follow up were statistically non-significant ($P > 0.05$). As shown in table 2, we found no statistically significant changes in manifest spherical equivalent from the baseline in the two groups ($P > 0.05$). Also, there was no statistically significant difference

between both groups in the manifest spherical equivalent at the 3rd and 6th months of the follow-up ($P > 0.05$).

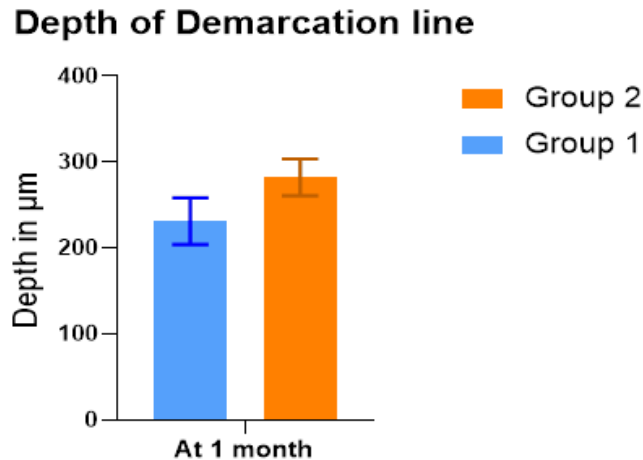


Fig. 3. The demarcation line depth (μm) in the 2 groups determined by AS-OCT at 1st month after the procedure (Group 1 = accelerated cross-linking $18 \text{ mW}/\text{cm}^2$ for 5 minutes ($5.4 \text{ J}/\text{cm}^2$); Group 2 = accelerated cross-linking $18 \text{ mW}/\text{cm}^2$ for 6 minutes and 40 seconds ($7.2 \text{ J}/\text{cm}^2$).

Table 2
Changes in CDVA and MRSE over six months postoperatively

Groups	Preoperative	3 rd month	6 th month	P-value*	P-value**
CDVA					
(Decimal)	0.59 ± 0.17	0.50 ± 0.19	0.58 ± 0.18	0.162	0.718
Group A	0.61 ± 0.16	0.48 ± 0.12	0.59 ± 0.15	0.681	
Group B					
MRSE (D)					
Group A	-3.12 ± 1.26	-3.05 ± 1.08	-3.17 ± 1.11	0.428	0.965
Group B	-3.08 ± 1.08	-3.07 ± 1.08	-3.15 ± 1.11	0.267	

CDVA: corrected distance visual acuity; MRSE: manifest refraction spherical equivalent; Group A = accelerated cross-linking $18 \text{ mW}/\text{cm}^2$ for 5 minutes ($5.4 \text{ J}/\text{cm}^2$); Group B = accelerated cross-linking $18 \text{ mW}/\text{cm}^2$ for 6 minutes and 40 seconds ($7.2 \text{ J}/\text{cm}^2$); D: diopter

* 6th month to preoperative (paired *t*-test)

** Intergroup differences at 6th-month follow-up (unpaired *t*-test)

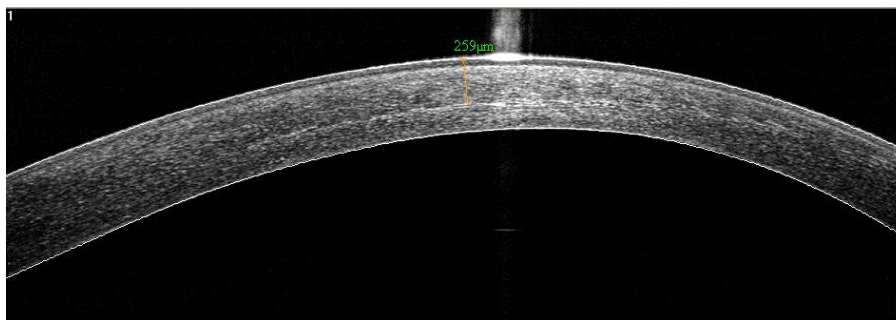


Fig. 4. AS-OCT of the patient number 7 in the second group (7.2 J/cm²) showing the DL depth at 259 μm

Keratometry values

Table 3 shows the mean changes in flat K (K1), steep K (K2), and maximum K at the 1st, 3rd, and 6th months of follow-up in both groups. We found statistically non-significant changes in the mean K1, K2, and Kmax at the 1st, 3rd, and 6th months. There was no statistically significant difference between both groups ($P > 0.05$) regarding the keratometry values.

Table 3
Changes in keratometric readings

Groups	Preoperative	1 st month	3 rd month	6 th month	P-value*	P-value**
K ₁ (D)						
Group A	45.519	45.409	45.395	45.352	0.342	0.961
Group B	±2.948	±2.870	±2.864	±2.869	0.452	
	45.285	45.460	45.475	45.405		
	±3.771	±4.016	±4.021	±3.995		
K ₂ (D)						
Group A	49.129	49.152	49.143	49.576	0.363	0.866
Group B	±2.95	±2.940	±2.949	±3.911	0.301	
	49.820	49.840	49.825	49.790		
	±4.083	±4.079	±4.170	±4.138		
K _{max} (D)						
Group A	54.700	54.623	54.609	54.642	0.076	0.674
Group B	±3.922	±3.951	±3.939	±3.917	0.555	
	55.300	55.250	55.445	55.390		
	±6.639	±6.633	±7.057	±7.011		

K₁: flat keratometry; K₂: steep keratometry; K_{max}: maximum keratometry; Group A = accelerated cross-linking 18 mW/cm² for 5 minutes (5.4 J/cm²); Group B = accelerated cross-linking 18 mW/cm² for 6 minutes and 40 seconds (7.2 J/cm²); D: diopter

* 6th month to preoperative (paired *t*-test)

** Intergroup differences at 6th-month follow-up (unpaired *t*-test)

Corneal densitometry

Total corneal densitometry values were increased from preoperative values (13.24±1.903 GSU), peaked at the 3rd month (17.30±1.991 GSU) in the first group and from (13.54±1.407 GSU) preoperatively to (17.55±1.837 GSU) at the 3rd month in the second group ($P < 0.05$ in both groups). We found no statistically significant difference between the two groups at the 1st, 3rd, and 6th months follow-up ($P > 0.05$), as shown in table 4. Corneal densitometry values in the anterior corneal layers were peaked at the 3rd month follow-up in both groups. In contrast, middle and posterior stromal layers peaked at the 6th month in both groups, as shown in figure 5. We found no statistical differences in mean changes in corneal densitometric values in all layers and zones between the two groups ($P > 0.05$). We reported the corneal densitometry values in all layers and zones remained significantly high at the 6th month following the procedure in both groups ($P < 0.05$ in both groups). We found no statistically significant correlation ($P > 0.05$) between total corneal densitometry values, MRSE ($r = 0.243$), and CDVA ($r = 0.344$) at the 6th month follow-up visit in the first group. Also, there was no statistically significant correlation ($P > 0.05$) between total corneal densitometry values, MRSE ($r = 0.245$), and CDVA ($r = -0.021$) at the 6th month follow-up visit in the second group.

Table 4
Corneal densitometry changes in stromal layers and concentric zones over time

Group	Preoperative	1 st month	3 rd month	6 th month	P-value*	P-value**
Total						
Group A	13.24 ±1.903	16.30	17.30	15.66	<0.05	0.619
Group B	13.54 ±1.407	±1.929 16.23 ±1.872	±1.991 17.55 ±1.837	±1.961 15.96 ±1.836	<0.05	
Anterior						
Group A	16.00 ±3.029	21.50	22.63	19.52	<0.05	0.396
Group B	16.50 ±2.484	±3.148 22.29 ±2.747	±3.656 23.95 ±2.446	±3.690 20.02 ±2.978	<0.05	
Middle						
Group A	12.62 ±2.061	15.59	15.13	16.69	<0.05	0.544
Group B	13.05 ±1.236	±2.026 16.09 ±1.520	±2.009 15.58 ±1.563	±2.010 17.04 ±1.607	<0.05	
Posterior						
Group A	11.06 ±1.651	12.26	11.80	14.80	<0.05	0.637
Group B	11.37 ±2.284	±1.709 12.64 ±2.250	±1.640 12.13 ±2.257	±1.644 15.11 ±2.351	<0.05	
0-2 mm						
Group A	11.88 ±2.752	16.51	18.42	18.98	<0.05	0.827
Group B	12.11 ±2.511	±3.083 16.96 ±2.823	±3.001 19.12 ±2.891	±3.109 19.21 ±3.538	<0.05	

2-6 mm						
Group A	11.06 ±2.306	15.28	14.75	13.90	<0.05	0.578
Group B	11.05 ±1.696	±2.558	±2.500	±2.489	<0.05	
		15.51	15.11	14.29		
		±1.960	±1.960	±1.970		
6-10 mm						
Group A	12.05 ±1.967	13.40	12.95	14.30	<0.05	0.164
Group B	12.56 ±1.879	±1.904	±1.923	±1.979	<0.05	
		14.06	13.48	15.17		
		±1.939	±1.918	±1.966		

Group A = accelerated cross-linking 18 mW/cm² for 5 minutes (5.4 J/cm²); Group B = accelerated cross-linking 18 mW/cm² for 6 minutes and 40 seconds (7.2 J/cm²); D: diopter
 * 6th month to preoperative (paired *t*-test)
 ** Intergroup differences at 6th-month follow-up (unpaired *t*-test)

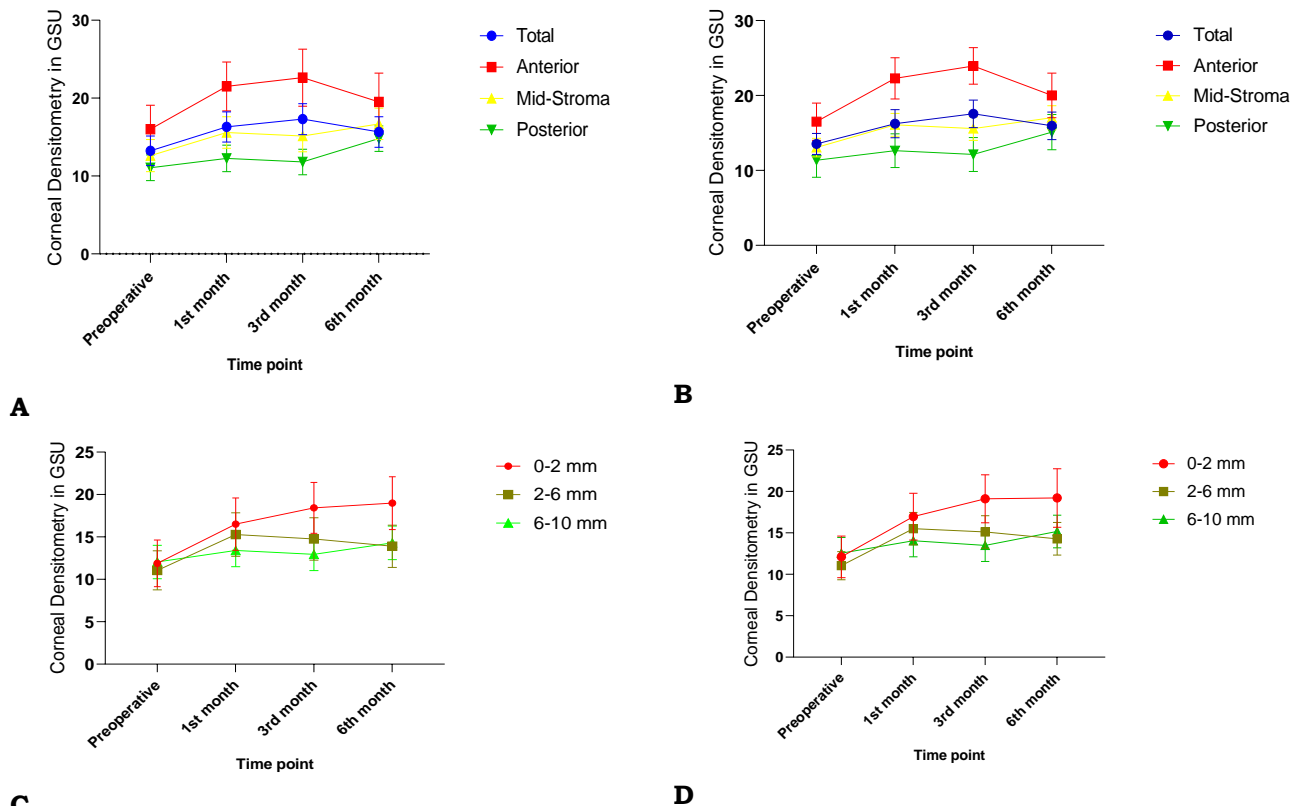


Fig. 5. Corneal densitometry changes in both groups: (A) Corneal densitometry changes in group A (5.4 J/cm²) measured in total, anterior, posterior, and mid-stromal layers over 6 months period following the procedure. (B) Corneal densitometry changes in group B (7.2 J/cm²) measured in total, anterior, posterior, and mid-stromal layers over 6 months period following the procedure. (C) Corneal densitometry changes in group A (5.4 J/cm²) measured 0-2, 2-6-, and 6-10-mm concentric zones over 6 months period following the procedure. (D) Corneal densitometry changes in group B (7.2 J/cm²) measured 0-2, 2-6-, and 6-10-mm concentric zones over 6 months period following the procedure

Corneal Haze Grading

Slit-lamp grading of corneal haze decreased from 1.62 ± 0.74 at the 1st month to 0.81 ± 0.60 at the sixth month in the first group and from 1.70 ± 0.66 to 0.70 ± 0.65 in the second group, and this reduction was statistically significant ($P < 0.05$ in both groups). We found no statistically significant difference between the two groups ($P > 0.05$).

Specular microscopy

We found that the mean endothelial cell number before the procedure was 2911.8 ± 120.17 cells/mm², 2977.85 ± 151.91 cells/mm² in group A and group B, respectively, and showed no significant change at the 6th month follow-up in both groups ($P > 0.05$ in both groups). We found no statistically significant difference between the two groups ($P > 0.05$).

Table 5
Corneal stromal demarcation line depth after CXL

Study	CXL Protocol (mW/cm ²)/Time (minutes)	Total energy (J/cm ²)	DL depth (μ m)
Kymionis ⁽¹³⁾	Group 1: 3 / 30	5.4	Group 1: 337 ± 46.46
	Group 2: 9 / 14	7.5	Group 2: 322.91 ± 48.28
Kymionis ⁽¹⁴⁾	Group 1: 3 / 30	5.4	Group 1: 341.81 ± 47.02
	Group 2: 18 / 7	7.5	Group 2: 313.37 ± 48.85
Tomita ⁽³⁾	Group 1: 3 / 30	5.4	Group 1: 380.78 ± 60.57
	Group 2: 30 / 3	5.4	Group 2: 294.38 ± 60.57
Mita ⁽¹⁵⁾	30 / 3	5.4	300
Ozgurhan ⁽¹⁶⁾	30 / 3	5.4	205.64 ± 18.5
Moramarco ⁽¹⁷⁾	Group 1: 30 / 4	7.2	Group 1: 149.32 ± 36.03
	Group 2: 30 / 8 (Pulsed)	7.2	Group 2: 213 ± 47.38
Spadea ⁽¹⁸⁾	10 / 9	5.4	279.34 ± 33.06
Böhm ⁽¹⁹⁾	30 / 8 (Pulsed)	7.2	203.00 ± 13.53
CXL: corneal cross-linking; DL; demarcation line			

Discussion

Accelerated cross-linking has been found to be equal to the standard cross-linking regarding corneal biomechanical response as well as clinical results. (3) Hashemi et al. compared A-CXL (18 mW/cm² UVA irradiance for five minutes) and standard cross-linking. They reported no difference between the two groups after six months and 18 months follow-up in stopping KC's progression. (8,9). We found in our study that the corneal stromal DL was shallower in the 5.4 J/cm² group (231.43 ± 27.18 μ m) compared to the 7.2 J/cm² group (282.3 ± 21.4 μ m) and

the difference was statistically significant ($P < 0.05$). According to published data, standard cross-linking shows a deeper demarcation line than accelerated cross-linking; this should not be misunderstood as a lower effect as the results of accelerated crosslinking regarding corneal stability proved the opposite. (4,10,11,12)

As shown in table 5, it is evident that less exposure time produces a more superficial DL. If the Bunsen-Roscoe law of reciprocity truly applies, the corneal stromal DL would theoretically not indicate the cross-linking effectiveness. We reported that the changes in the CDVA were not statistically significant ($P > 0.05$). Also, there were no statistically significant changes in manifest spherical equivalent from the baseline in both groups. Additionally, there was no difference between the two groups in the MRSE at the 3rd and 6th months of the follow-up ($P > 0.05$). We recorded the mean changes in K1, K2, and maximum K at the 1st, 3rd, and 6th months of follow-up in both groups. We found no statistically significant changes in the mean K1, K2, and Kmax at the 1st, 3rd, and 6th months. There was no statistically significant difference regarding keratometry values between the two groups ($P > 0.05$).

In accordance with our study, Hashemi et al. found that the changes in the CDVA, MRSE, and keratometric values were not statistically significant at the 6th and 18th months follow-up using a power of 18 mW/cm² for 5 minutes. (8,9) Gatzoufas et al. reported no significant changes in CDVA, Kmax, mean K values in the 6th month follow-up. (20). Alnawaiseh et al. found no statistically significant changes in CDVA, mean K, K1, and K2 after a mean follow-up period of 21.7 ± 7.2 months. They reported a significant decrease in Kmax. (21). Shetty et al. conducted a study using 4 different CXI protocols: group 1: Dresden protocol; group 2: 9 mW/cm² for 10 minutes; group 3: 18 mW/cm² for 5 minutes; group 4: 30 mW/cm² for 3 minutes. They found a significant improvement in CDVA and SE in all groups except group 4. They also reported a significant flattening of K1 and K2 in the first two groups. (22)

A recent study found that A-CXL with a modified higher UVA intensity might produce corneal stromal haze during the initial postoperative phase. (23). Subjective assessment of the corneal haze is difficult over time, especially with different observers. A Scheimpflug corneal imaging system introduced a special densitometry software, allowing objective and quantitative assessment of corneal haze. (7). We reported that the corneal densitometry was peaked in the anterior corneal layers at the 3rd month follow-up in both groups. In contrast, the middle and posterior stromal layers peaked at the 6th month in the two groups. The most remarkable change in densitometry values was detected in the anterior stromal layer, especially in the most central zones (0.0-2.0 mm and 2.0-6.0 mm). These findings are similar to the results of Pircher et al. They evaluated corneal light scattering using corneal densitometry after the Dresden protocol. They found that changes in the corneal densitometry were maximum at the 3rd month in all three layers. At one year, the middle and posterior layers returned nearly to the baseline values, while the anterior layer values remained higher than baseline values. They reported no significant correlation between CDVA and corneal densitometry values ($r < 0.3$). (24)

The densitometry's remarkable changes in the anterior stromal layers may be attributed to the decline of oxygen concentration, UVA irradiance intensity, and riboflavin concentration toward deeper stromal layers. (19). We found no significant correlation between changes in total densitometry, CDVA, and MRSE in both groups ($P>0.05$). Böhm et al. analyzed the corneal densitometry changes following modified A-CXL (30 mW/cm² UVA irradiance for 8 minutes (pulsed protocol)). They found that the corneal densitometric values increased in the three corneal stromal layers at the 3rd month compared to preoperative values. The increased mean densitometry in the anterior stromal layer was statistically significant ($P<0.05$), whereas the changes in the middle and posterior layers were not significant. (19)

Mathews et al. conducted a study to assess corneal haze using corneal densitometry and correlate it with the visual outcomes following the Dresden protocol. They reported that the total corneal densitometry, anterior, and mid-stromal values were increased significantly and peaked at the 3rd month ($P<0.05$). They found a strong correlation between densitometry changes in the mid-stromal 2.0-6.0 mm annulus and CDVA at six months ($P<0.05$) and 24 months ($P<0.05$). They also reported a weak correlation between CDVA and changes in densitometry values in the total mid-stromal layer at six months ($P<0.05$). (25). Turhan et al. compared the changes in corneal densitometry after two A-CXL protocols. In both groups (5.4 J/cm² and 7.2 J/cm²), they found that the mean densitometry values increased significantly and peaked at the 1st month and returned to the baseline values at the 12th month follow-up. (23)

In a recent study, Hafezi et al. reported a significant increase in the anterior (+3.12±3.36 GSU), central (+1.97±2.09 GSU), posterior (+0.90±1.47 GSU), and total (+2.00±2.07 GSU) corneal densitometry after 12 months following modified cross-linking (the Sub400 protocol). (26). We found no significant loss of endothelial cells at the 6th month follow-up in both groups in the current study regarding corneal endothelium. Similarly, Gatzoufas et al. and Shetty et al. reported no statistically significant changes in endothelial cell density. (20,27). The A-CXL may entail less harmful effects on the endothelial cells due to its shallower effect than Dresden protocol. The studies have confirmed this hypothesis and showed that endothelial cell loss was not significant. (15,20,27,28). Different studies confirmed the safety of modified cross-linking with increased total delivered energy (7.2 J/cm² or 7.5 J/cm²) even after a long follow-up period of 24 months. (10,13,27). In conclusion, we found that accelerated cross-linking and modified accelerated cross-linking protocols were effective and safe in halting keratoconus progression after a follow-up period of 6 months. However, in the group with modified A-CXL, we obtained a deeper corneal stromal DL by increasing the total delivered energy to the cornea from 5.4 J/cm² to 7.2 J/cm². Regarding corneal densitometry, the changes induced by corneal cross-linking seemed to be more profound in the anterior 120 µm of the cornea and the 0.0-2.0 mm central zone. This alteration in the corneal transparency has no significant effect on CDVA.

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