Polskie Towarzystwo Okulistyczne
www.pto.com.pl
KLINIKA OCZNA 2023, 125, 3: 151-157
Received: 19.03.2022 Accepted: 5.06.2022

ORIGINAL ARTICLE

Access this article online
Website: www.klinikaoczna.pl
DOI: https://doi.org/10.5114/ko.2023.131906

Corneal changes in adult patients with progressive keratoconus after accelerated corneal cross-linking: prospective 6-month interim study results

Tomas Mickevicius, Ugne Rumelaitiene, Renata Vaiciuliene, Aleksandra Kubiliute, Dalia Zaliuniene

Department of Ophthalmology, Lithuanian University of Health Sciences, Kaunas, Lithuania

ABSTRACT

Aim of the study: To assess corneal densitometry (CD) and endothelium changes in adult patients with progressive keratoconus (PKC) after accelerated corneal cross-linking (A-CXL) and compare them with healthy myopic controls.

Material and methods: 12 PKC patients who underwent A-CXL between 5 August 2020 and 22 March 2021 were examined and compared with 12 healthy myopes (control group). Evaluation of visual acuity, IOP, corneal topography, densitometry and specular microscopy were performed preoperatively and at the 1st, 3rd and 6th postoperative month.

Results: In the PKC group, a significant elevation of CD values were found in the ∅ 0-2, ∅ 2-6 zones of the anterior, central and total corneal layers after each follow-up visit. In the posterior corneal layer, they were significantly higher only in the ∅ 2-6 zone at the 6th postoperative month. The mean CD values of myopic corneas was statistically significantly lower only in the ∅ 0-2 and ∅ 2-6 zones of the anterior, central and total layers compared with PKC-affected corneas 6 months after the A-CXL procedure. In the posterior corneal layer, the mean CD values of myopes were statistically significantly lower only in the ∅ 2-6 zone compared with baseline and 6-month post-A-CXL values. A statistically significant decrease of the endothelial coefficient of variation and increase of number of hexagonal cells was found 6 months after A-CXL in the PKC group compared with controls.

Conclusions: A-CXL induces changes in corneal transparency, especially in the central 6 mm zone of anterior and central corneal stromal layers. Furthermore, transient morphological modifications in the corneal endothelium may occur following this procedure.

KEY WORDS: corneal cross-linking, keratoconus, corneal densitometry.

INTRODUCTION

The cornea is an avascular and transparent structure of the eye. It consists of five separate layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. Each of its layers has a specific function and any change in their structure results in consequential corneal disorders [1].

Thinning of the cornea is a characteristic feature of ectatic disorders. The most common one is keratoconus (KC) [2]. A wide range of histopathological studies have revealed which abnormalities are usually found in the KC-affected corneal tissues. These include the enlargement of basal epithelial cells, their irregular arrangement and a significantly lowered cell density in comparison to normal corneas [3-5]. The ruptures in Bowman's layer [6] and morphological folds of Descemet's membrane [7] were also described as a characteristic feature of KC-affected corneas. The most apparent changes are found in the corneal stroma which is known to have a significantly decreased number of anterior lamellae [8, 9] and stromal keratocytes [4, 6, 10-12]. Findings in the corneal endothelium are controversial. While some studies suggest an increase in endothelial cell density [13], others show a significant decrease in moderate-to-severe KC cases [7, 10].

The corneal cross-linking (CXL) procedure was introduced to halt the progression of KC [14]. It improves the distribution of the collagen fibrils, increases their diameter and interfibrillar spacing in KC-affected corneas. Furthermore, the mean proteoglycan area becomes smaller after CXL is performed [15]. However, this procedure carries a risk of the development of corneal haze or scarring [16, 17].

Corneal changes after CXL can be evaluated using corneal imaging tools. Pentacam®HR (Oculus Inc., Wetzlar, Germa-

CORRESPONDING AUTHOR
Renata Vaiciuliene, Department of Ophthalmology, Lithuanian University of Health Sciences Kauno Klinikos, Eiveniu 2, LT-50161 Kaunas, Lithuania; e-mail: renata.vaiciuliene@lsmuni.lt

ny) is a noninvasive optical system that uses the Scheimpflug principle to assess the anterior segment of the eye. It provides complete information about the corneal thickness, topography and transparency [18, 19]. Specular microscopy (NIDEK, USA; Specular Microscope CEM-530) is another non-invasive corneal imaging tool that allows one to perform a quantitative analysis of corneal endothelium [20].

The purpose of this study was to objectively evaluate the impact of accelerated CXL (A-CXL)-induced changes on corneal transparency and endothelium.

MATERIAL AND METHODS

Patients and selection

We performed a prospective, longitudinal, non-randomized study. We included all patients with progressive KC (PKC) who underwent the A-CXL procedure in the period from 5 August 2020 to 22 March 2021 performed by 1 surgeon (U.R.) in the Department of Ophthalmology, Hospital of Lithuanian University of Health Sciences Kauno Klinikos (Kaunas, Lithuania) and healthy age-, sex- and eye-matched myopic controls. The study protocol was reviewed and approved by Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-63, 23/04/2020). Inclusion criteria for the PKC group were age above 18 years and being diagnosed with PKC. A diagnosis of PKC was defined as an increase of the maximum K-value (K_{max}) by at least 1 diopter (D), decrease of the thinnest corneal thickness (TCT) by at least 30 µm, an increase of cylinder by at least 1 D, an increase of manifest refraction spherical equivalent (MRSE) by at least 0.50 D or more in over a 12-month observation period [21]. The control group consisted of healthy myopic age-, sex- and eye-matched study participants. Informed written consent was obtained from all eligible study participants after a detailed explanation of the ongoing study in accordance with the Declaration of Helsinki. Exclusion criteria for both groups were history of ocular trauma, current systemic or ocular disease and the refusal to sign the informed written consent. Patients in the PKC group were not included if there were any contraindications to perform the A-CXL procedure.

Preoperative ophthalmologic examination

All study participants and controls underwent a standard ophthalmic examination in an outpatient clinic performed by the same ophthalmologist (U.R.) in the Department of Ophthalmology, Hospital of Lithuanian University of Health Sciences Kauno Klinikos (Kaunas, Lithuania), including uncorrected distance visual acuity (UDVA) and best corrected visual acuity (BCVA) evaluation, IOP measurement (iCare TA01i, Icare Finland Oy, Vantaa, Finland), slit-lamp examination, Pentacam® analysis (corneal topography and densitometry), and specular microscopy for the evaluation of endothelium. The assessment of UDVA and BCVA was performed using logMAR units 4 meters from the participant under photopic conditions.

Surgical procedure

Only patients with PKC were scheduled to undergo a 10-minute "epithelium-off" A-CXL [22]. All procedures were performed in a single center (Department of Ophthalmology, Hospital of Lithuanian University of Health Sciences Kauno Klinikos) by the same surgeon (U.R.) under local anesthesia. First a lid speculum was inserted and the central 9 mm zone of corneal epithelium was debrided. Then an isotonic solution of 0.1% riboflavin (MEDIOCROSS® M, Avedro, Waltham, MS, USA) every 2 minutes for 30 minutes was instilled. The central corneal thickness of the treated eye was then measured with an ultrasonic pachymeter (Tomey Bio and Pachymeter AL-4000, Nuremberg, Germany) to ensure it has a minimum of 400 µm prior to UV irradiation. UVA light (375 nm) (UV-X™ 2000 Crosslinking System, Avedro, Waltham, MS, USA) was used to irradiate the cornea for 10 minutes at 9 mW/cm^2 irradiance (5.4 J/cm^2 total energy). During the irradiation, the riboflavin solution was administered every 2 minutes. At the end of each procedure, one drop of levofloxacin was administered and a soft bandage contact lens was applied to the treated eye.

Postoperative care and follow-up ophthalmologic examinations

After each procedure patients with PKC received standard care with levofloxacin 0.5% (4 times daily for 2 weeks) and dexamethasone 0.1% (twice daily for the first 5 days and then tapering it down by 1 drop weekly) drops. Patients were also advised to use preservative-free artificial tears and vitamin C supplements. Soft bandage contact lenses were removed after full epithelialization of the cornea (5 days after the procedure). Subsequent postoperative outpatient follow-up visits were scheduled 1 (35 ±5 days), 3 (94 ±4 days) and 6 (189 ±20 days) months after A-CXL. Data about patients' UDVA, BCVA, IOP, corneal topographic and densitometry values were recorded. Corneal densitometry (CD) was assessed at the anterior (120 µm front), central, posterior (60 µm rear) and total layers and their central 2 (∅ 0-2) mm, radial 2-6 (∅ 2-6) mm, radial 6-10 (∅ 6-10) mm and radial 10-12 (∅ 10-12) mm zones. Its values are expressed in grayscale units (GSUs), which define backward light scatter and range from 0 (maximum transparency) to 100 (completely opaque). Specular microscopy was also performed for each study participant to evaluate postoperative changes in endothelial cell density (ECD), the coefficient of variation (CV) and the number of hexagonal cells (6A) at each follow-up visit.

Statistical analysis

The SPSS Statistics (IBM) software platform (version 27.0) was used for the statistical analysis of the gathered data. Descriptive statistics (mean, standard deviation [SD] and percent) were used to summarize the baseline data. The Kolmogorov-Smirnov Z test was used to assess the normal distribution. Comparisons of UDVA, BCVA, IOP, TCT, and K-values were performed against baseline at each follow-up

point using non-parametric Mann-Whitney U test or Wilcoxon's signed-rank tests, as appropriate. Repeated measures analyses of variance (RM-ANOVA) with Bonferroni adjusted post-hoc comparisons were performed to evaluate CD, as well as values of ECD, CV and 6A preoperatively and 1, 3 and 6 months after A-CXL. Multifactor ANOVA with Bonferroni adjusted post-hoc comparisons was chosen to perform the assessment of the difference between the CD and ECD, CV and 6A values of the baseline, 6-month postoperative PKC group and control group. For the assessment of the correlation of CD values with UDVA, BCVA, TCT, K-values, ECD, CV and 6A Pearson correlation coefficients (r) were calculated. P-values less than 0.05 were considered statistically significant.

RESULTS

Patients' demographics and baseline data of corneal parameters

The baseline data of study participants' demographic and corneal parameters are shown in Table I. All patients completed a 6-month postoperative follow-up. The mean values of BCVA, IOP and TCT in the PKC group were significantly lower than in the control group. The mean preoperative corneal K-values (K_{steep}, K_{flat} and K_{max}) were statistically significantly higher in the PKC group compared with controls.

Table I. Patients' demographics and baseline data

Variables	KC group ($N = 12$) Mean ±SD	Control group ($N = 12$) Mean ±SD	p value
Age, years	25.33 ±7.54	25.33 ±7.54	1.0
UDVA, logMAR	0.88 ±0.36	0.81 ±0.36	0.561
BCVA, logMAR	0.48 ±0.29	0	**< 0.001**
MRSE, D	–3.77 ±3.41	–3.31 ±1.53	0.795
IOP, mmHg	9.68 ±1.88	12.08 ±2.19	**0.008**
K_{steep}, D	51.77 ±4.22	43.78 ±1.62	**< 0.001**
K_{flat}, D	46.75 ±3.00	42.83 ±1.47	**0.001**
K_{max}, D	59.53 ±6.13	44.26 ±1.52	**< 0.001**
TCT, µm	454.42 ±30.39	527.58 ±25.40	**< 0.001**
Corneal endothelium			
ECD, cells/mm^2	2941.08 ±263.07	2924.75 ±325.09	0.840
CV	32.00 ±5.59	29.08 ±3.40	0.172
6A, %	61.67 ±6.33	65.83 ±5.41	0.063

KC – keratoconus; UDVA – uncorrected distant visual acuity; BCVA – best corrected visual acuity; TCT – thinnest corneal thickness; CD – cell density; CV – coefficient of variation; 6A – number of cells that have a hexagonal shape; MRSE – manifest refraction spherical equivalent

Visual acuity and intraocular pressure

Six-month follow-up postoperative results revealed a statistically significant improvement of both UDVA and BCVA compared with the preoperative data. In the PKC group, 7 out of 12 (58%) patients' UDVA improved, for 3 out of 12 (25%) it remained stable, and for 2 patients (17%) it decreased by at least one line. Furthermore, 7 out of 12 (58%) patients' BCVA improved, for 4 out of 12 (33%) it remained stable and for 1 patient (9%) it decreased by at least one line.

At the 6th postoperative month, in the PKC group the mean value of IOP was non-significantly lower (9.59 ±2.74 mmHg) compared with the mean preoperative (9.68 ±1.88 mmHg) results ($p = 1.0$).

Corneal topography and thickness

Figure 1 shows changes in the mean values of main keratometry readings in the PKC group after A-CXL. K_{max} was statistically significantly lower at the 3rd ($p = 0.023$) and 6th ($p = 0.003$) postoperative month compared to the baseline data, whereas K_{steep} and K_{flat} values were statistically significantly higher after 1st ($p = 0.008$ and $p = 0.002$) postoperative month but became statistically significantly lower at the 6th ($p = 0.009$ and $p = 0.034$) postoperative month compared with the baseline results.

TCT in the PKC group was statistically significantly lower at the 6th postoperative month (437.25 ±28.38 µm) compared to the preoperative (454.42 ±30.39 µm) data ($p = 0.004$).

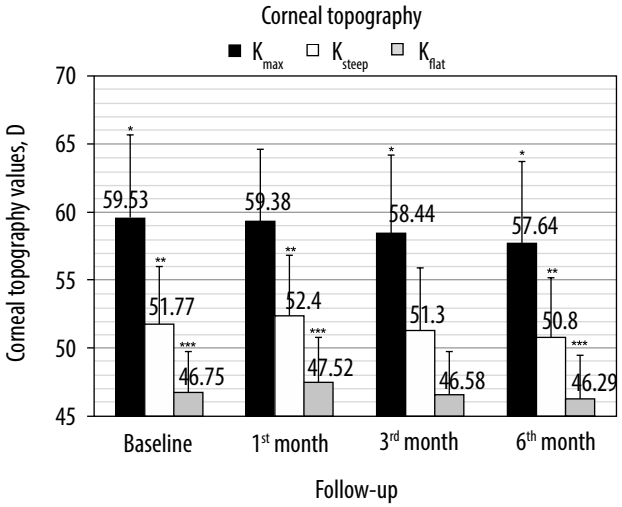

Figure 1. Changes in the mean values of main keratometry readings in the KC group. Statistically significant difference between *K_{max}, **K_{step} and ***K_{flat} values

Corneal densitometry

The analysis of the mean CD values in the PKC group revealed that they were statistically significantly higher in the ∅ 0-2, ∅ 2-6 zones of the anterior, central and total corneal layers after each follow-up visit compared with the baseline results (Table II). They were statistically significantly higher in the ∅ 6-10 zone of anterior, central, posterior and total corneal layers only after the 1st postoperative month (all $p < 0.05$). In the posterior corneal layer, a significant increase of the mean CD values was observed in the ∅ 0-2 zone but only at the 3rd postoperative month. At the 6th postoperative month, in the same corneal layer mean CD values were sta-

Table II. Corneal densitometry values in KC group after accelerated CXL ($n = 12$)

	Baseline	1st month	3rd month	6th month
Anterior				
0-2	22.73 ±0.44	33.09 ±1.69	34.29 ±1.90	35.00 ±2.96
2-6	20.52 ±0.25	28.63 ±0.91	27.80 ±0.94	25.12 ±0.98
6-10	18.93 ±0.80	22.33 ±0.68	20.72 ±1.05	19.42 ±0.76
10-12	26.48 ±2.08	26.97 ±2.50	27.47 ±2.54	27.55 ±2.78
Central				
0-2	14.83 ±0.18	18.87 ±0.90	19.10 ±1.00	18.80 ±0.96
2-6	13.03 ±0.13	17.36 ±0.61	16.17 ±0.70	15.14 ±0.42
6-10	13.47 ±0.56	15.24 ±0.59	14.38 ±0.71	13.74 ±0.54
10-12	18.08 ±1.33	19.18 ±1.68	19.14 ±1.46	18.89 ±1.42
Posterior				
0-2	7.08 ±0.24	8.44 ±0.49	8.66 ±0.41	8.38 ±0.26
2-6	8.78 ±0.21	10.85 ±0.57	10.37 ±0.60	10.02 ±0.33
6-10	10.74 ±0.50	11.77 ±0.59	11.58 ±0.67	11.12 ±0.55
10-12	15.86 ±1.05	16.93 ±1.34	16.81 ±1.04	17.02 ±1.23
Total				
0-2	14.88 ±0.25	20.13 ±0.99	20.68 ±1.01	20.71 ±1.29
2-6	14.10 ±0.14	18.94 ±0.61	18.10 ±0.64	16.77 ±0.50
6-10	14.39 ±0.61	16.44 ±0.59	15.56 ±0.79	14.76 ±0.59
10-12	20.16 ±1.39	21.03 ±1.79	21.14 ±1.64	21.15 ±1.75

	Baseline vs. 1st month	Baseline vs. 3rd month	Baseline vs. 6th month
Anterior			
0-2	$p = 0.001$	$p = 0.001$	$p = 0.010$
2-6	$p < 0.001$	$p < 0.001$	$p = 0.005$
6-10	$p < 0.001$	$p = 0.084$	$p = 0.317$
10-12	$p = 1.0$	$p = 1.0$	$p = 1.0$
Central			
0-2	$p = 0.009$	$p = 0.011$	$p = 0.017$
2-6	$p < 0.001$	$p = 0.008$	$p = 0.004$
6-10	$p < 0.001$	$p = 0.210$	$p = 0.375$
10-12	$p = 1.0$	$p = 1.0$	$p = 1.0$
Posterior			
0-2	$p = 0.188$	$p = 0.023$	$p = 0.057$
2-6	$p = 0.014$	$p = 0.146$	$p = 0.043$
6-10	$p = 0.004$	$p = 0.490$	$p = 0.986$
10-12	$p = 0.250$	$p = 1.0$	$p = 0.090$
Total			
0-2	$p = 0.004$	$p = 0.002$	$p = 0.007$
2-6	$p < 0.001$	$p = 0.001$	$p = 0.002$
6-10	$p < 0.001$	$p = 0.161$	$p = 0.449$
10-12	$p = 1.0$	$p = 1.0$	$p = 1.0$

tistically significantly higher only in the ∅ 2-6 zone. After an entire follow-up period, we also found a statistically significant decrease of the mean CD values in the ∅ 2-6 and ∅ 6-10 zones of the anterior ($p = 0.019$ and $p < 0.001$), central ($p = 0.003$ and $p = 0.003$) and total ($p = 0.008$ and $p < 0.001$) corneal layers compared with the values at the 1st postoperative month.

The mean CD values of myopic corneas were statistically significantly lower only in the ∅ 0-2 and ∅ 2-6 zones of the anterior, central and total layers compared with PKC-affected corneas 6 months after A-CXL procedure (Figure 2). In the posterior corneal layer, the mean CD values of myopes were statistically significantly lower only in the ∅ 2-6 zone compared with baseline ($p = 0.004$) and 6-month post-A-CXL values ($p < 0.001$).

Endothelium

In the PKC group, at the 6th postoperative month there were no statistically significantly changes in ECD, CV and 6A values compared with the preoperative results (Table III). No significant difference was found after comparing mean ECD values before the procedure, 6 months after A-CXL and controls (all $p > 0.05$). Nonetheless, there was a statistically significant difference when comparing the 6-month postoperative mean values of CV and 6A with controls ($p = 0.042$ and $p = 0.025$, respectively).

Interrelationship between corneal densitometry, visual acuity and other corneal parameters

No significant association between CD values of anterior, central and total layers and UDVA, BCVA, TCT, K-values, ECD, CV and 6A was found at the 6th postoperative month (all $p > 0.05$).

DISCUSSION

The 60-minute period of corneal exposure during the standard CXL (S-CXL) procedure carries an increased risk of complications [23]. This led to the search for alternative treatment protocols which would produce equally effective and safe results but would allow surgeons to reduce the time spent in the operating theatre. Several A-CXL protocols have been proposed which follow the photochemical law of reciprocity – the same photochemical effect can be achieved with reduced illumination time and increased irradiation intensity [24]. Kobashi *et al.* [25] found that A-CXL produce equally effective and safe outcomes regarding K_{max}, central corneal thickness, UDVA, corneal hysteresis, corneal resistance factor and endothelial cell density.

One of the most common early findings in CXL-treated corneas is transient corneal haze [26, 27]. Various hypotheses have previously been proposed to explain this phenomenon. Disarrangement in corneal morphology is considered as the most probable cause of corneal densitometry elevation [28-33]. Additionally, damage to keratocytes has been linked with CXL treatment and their transformation to myofibroblasts which are also thought to cause corneal

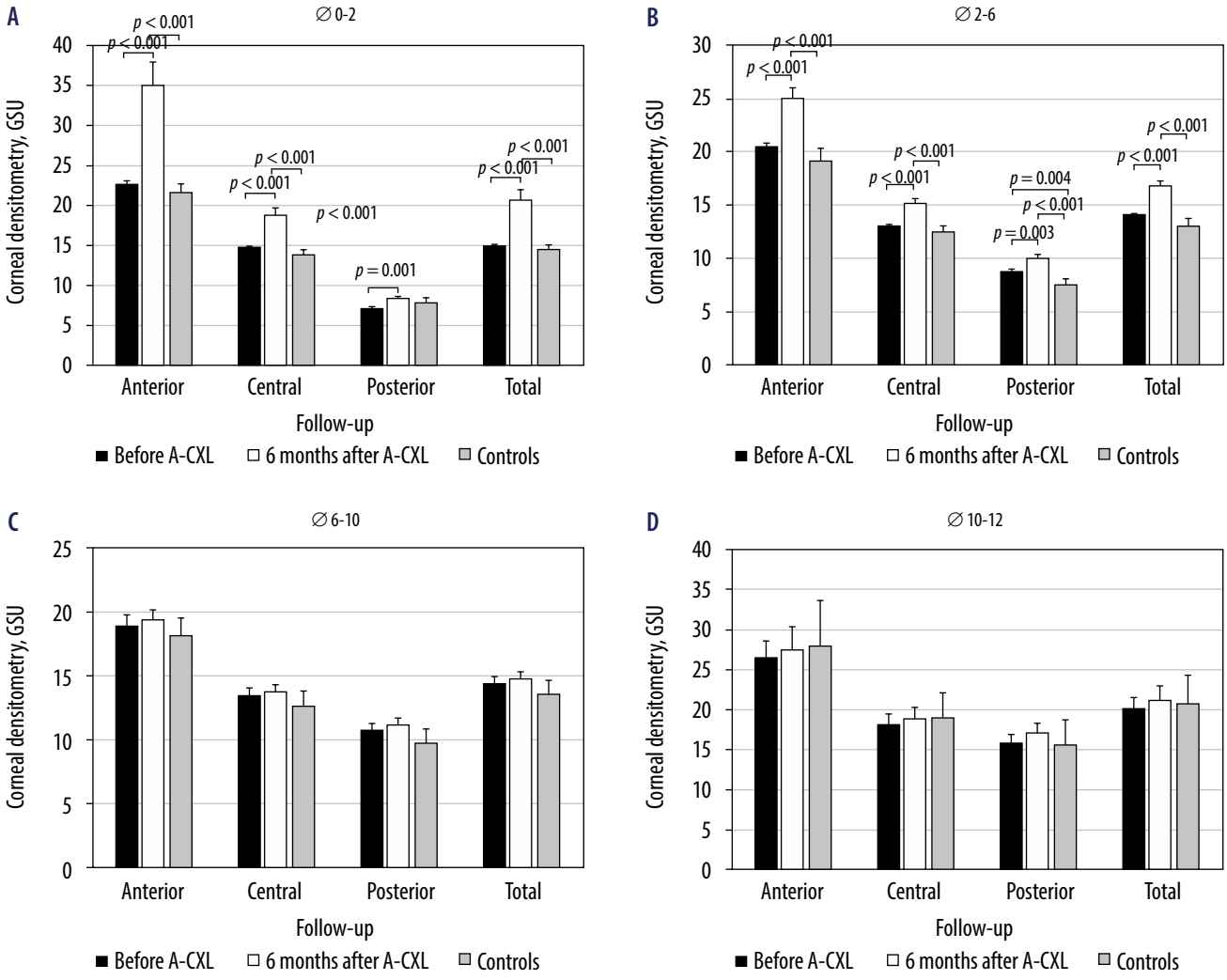

Figure 2. Comparison of mean corneal densitometry values in the 0-2 mm corneal zone, 2-6 mm corneal zone, 6-10 mm corneal zone and 10-12 corneal zone

Table III. Changes in Endothelium in KC Group ($n = 12$)

	Baseline	1st month	3rd month	6th month	*p*-value
CD (cells/mm^2)	2941.08 ±263.07	2914.83 ±347.97	2967.00 ±267.34	2879.33 ±191.64	(1) 0.638 (3) 0.754 (6) 0.583
CV	32.00 ±5.59	35.25 ±9.03	36.83 ±7.63	34.25 ±5.36	(1) 0.753 (3) 0.157 (6) 0.208
6A (%)	61.67 ±6.33	58.33 ±4.58	58.50 ±6.88	59.17 ±5.70	(1) 0.119 (3) 0.239 (6) 0.208

CD – cell density; CV – coefficient of variation; 6A – number of cells that have a hexagonal shape; (1) – baseline vs. 1st month; (3) baseline vs. 3rd month; (6) baseline vs. 6th month

stroma remodeling [34, 35]. Therefore, here we objectively evaluated the impact of CXL-induced changes on corneal transparency.

In this study, we used a 10-minute A-CXL protocol when a 10-minute irradiation at 9 mW/cm^2 was performed while delivering 5.4 J/cm^2 of energy. A significant increase of the mean CD values was mainly found in the ∅ 0-2 mm and ∅ 2-6 mm zones of the anterior, central and total layers at all follow-up times. These findings are partly in line with other studies that evaluated changes in CD after CXL procedure performed following accelerated protocols. Alzahrani *et al.* [36] in their prospective, cross-sectional study found a significant increase of mean CD values at the anterior and central layers of the corneal ∅ 0-2 mm and ∅ 2-6 mm zones

in the adult group after a 3-month follow-up. However, after that point they started to decrease and at the 6th postoperative month an increase found at that time point was not significant. Bohm *et al*. [37] performed a study where they evaluated changes in CD after performing a 4-minute UVA irradiation at 30 mW/cm^2 using 5.4 J/cm^2 of energy during A-CXL procedure. After a 3-month follow-up, they found an increase of CD values in all three corneal layers but it was significant only in the anterior (front 120 μm) portion. Interestingly, Shen *et al*. [38] found a significant decrease in CD values in central, posterior and total corneal layers after performing a 320-second the A-CXL at 45 mW/cm^2 using 7.2 J/cm^2 of energy. In contrast to the aforementioned studies, we found a significantly reduced corneal transparency in the ∅ 6-10 mm zone in all corneal layers but only after first postoperative month.

In our study, we also objectively evaluated A-CXL-induced endothelial changes after performing A-CXL. After a 6-month follow-up, we found that the mean of ECD in the PKC group decreased by 1.5%, 6A decreased by 4.8% and CV increased by 5.9%. Nonetheless, these changes were non-significant when comparing them with the preoperative results. After performing a 9-minute A-CXL at 10 mW/cm^2 using 5.4 J/cm^2 of energy, Badawi *et al*. [39] also found a non-significant decrease of 6A and increase of CV at the 6th postoperative follow-up month. In contrast, a significant decrease of ECD was found. When comparing these results with controls, we found a significantly higher CV and lower 6A. Our findings are in accordance with a study by Cingu *et al*. [40] where they performed a 5-minute A-CXL at 18 mW/cm^2 and found an increase of CV and decrease of 6A after comparing CXL-treated eyes with controls but they were non-significant.

There are two main limitations of this study. First, a follow-up period of 6 months was relatively short. Second, the sample size was small. Therefore, further research with larger sample size and longer follow-up is required.

CONCLUSIONS

CD values in the anterior and central corneal layers remain increased 6 months after a 10-minute A-CXL is performed, thus impacting the overall corneal transparency change. Furthermore, morphological modifications in corneal endothelial polymegethism and pleomorphism may occur following a 10-minute A-CXL procedure.

DISCLOSURE

The authors declare no conflict of interest.

References

1. Eghrari AO, Riazuddin SA, Gottsch JD. Overview of the Cornea: Structure, Function, and Development. Prog Mol Biol Transl Sci 2015; 134: 7-23.
2. Gordon-Shaag A, Millodot M, Shneor E, et al. The genetic and environmental factors for keratoconus. Biomed Res Int 2015; 2015: 795738.
3. Bitirgen G, Ozkagnici A, Bozkurt B, et al. In vivo corneal confocal microscopic analysis in patients with keratoconus. Int J Ophthalmol 2015; 8: 534-539.
4. Mocan MC, Yilmaz PT, Irkec M, et al. In vivo confocal microscopy for the evaluation of corneal microstructure in keratoconus. Curr Eye Res 2008; 33: 933-939.
5. Weed KH, MacEwen CJ, Cox A, et al. Quantitative analysis of corneal microstructure in keratoconus utilising in vivo confocal microscopy. Eye (Lond) 2007; 21: 614-623.
6. Sykakis E, Carley F, Irion L, et al. An in depth analysis of histopathological characteristics found in keratoconus. Pathology 2012; 44: 234-239.
7. Uçakhan OO, Kanpolat A, Ylmaz N, et al. In vivo confocal microscopy findings in keratoconus. Eye Contact Lens 2006; 32: 183-191.
8. Morishige N, Wahlert AJ, Kenney MC, et al. Second-harmonic imaging microscopy of normal human and keratoconus cornea. Invest Ophthalmol Vis Sci 2007; 48: 1087-1094.
9. Takahashi A, Nakayasu K, Okisaka S, et al. [Quantitative analysis of collagen fiber in keratoconus]. Nippon Ganka Gakkai Zasshi 1990; 94: 1068-1073.
10. Erie JC, Patel SV, McLaren JW, et al. Keratocyte density in keratoconus. A confocal microscopy study (a). Am J Ophthalmol 2002; 134: 689-695.
11. Mathew JH, Goosey JD, Bergmanson JP. Quantified histopathology of the keratoconic cornea. Optom Vis Sci 2011; 88: 988-997.
12. Niederer RL, Perumal D, Sherwin T, et al. Laser scanning in vivo confocal microscopy reveals reduced innervation and reduction in cell density in all layers of the keratoconic cornea. Invest Ophthalmol Vis Sci 2008; 49: 2964-2970.
13. Lema I, Durán JA. Inflammatory molecules in the tears of patients with keratoconus. Ophthalmology 2005; 112: 654-659.
14. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003; 135: 620-627.
15. Hayes S, Boote C, Kamma-Lorger CS, et al. Riboflavin/UVA collagen cross-linking-induced changes in normal and keratoconus corneal stroma. PLoS One 2011; 6: e22405.
16. Razmjoo H, Rahimi B, Kharraji M, et al. Corneal haze and visual outcome after collagen crosslinking for keratoconus: A comparison between total epithelium off and partial epithelial removal methods. Adv Biomed Res 2014; 3: 221.
17. Raiskup F, Kissner A, Hoyer A, et al. [Corneal scar development after cross-linking in keratoconus]. Ophthalmologe 2010; 107: 837-842.
18. Cho YK, Chang HS, La TY, et al. Anterior segment parametersusing Pentacam and prediction of corneal endothelialcell loss after cataract surgery. Korean J Ophthalmol 2010; 24: 284-290.
19. Otri AM, Fares U, Al-Aqaba MA, et al. Corneal densitometryas an indicator of corneal health. Ophthalmology 2012; 119: 501-508.
20. Chaurasia S, Vanathi M. Specular microscopy in clinical practice. Indian J Ophthalmol 2021; 69: 517-524.

21. Mohammadpour M, Masoumi A, Mirghorbani M, et al. Updates on corneal collagen cross-linking: Indications, techniques and clinical outcomes. J Curr Ophthalmol 2017; 29: 235-247.
22. Elbaz U, Shen C, Lichtinger A, et al. Accelerated (9-mW/cm^2) corneal collagen crosslinking for keratoconus-A 1-year follow-up. Cornea 2014; 33: 769-773.
23. Medeiros CS, Giacomin NT, Bueno RL, et al. Accelerated corneal collagen crosslinking: Technique, efficacy, safety, and applications. J Cataract Refract Surg 2016; 42: 1826-1835.
24. Bunsen RW, Roscoe HE. Photochemical researches – Part V. On the measurement of the chemical action of direct anddiffuse sunlight. Proc R SocLond 1862; 12: 306-312.
25. Kobashi H, Tsubota K. Accelerated Versus Standard Corneal Cross-Linking for Progressive Keratoconus: A Meta-Analysis of Randomized Controlled Trials. Cornea 2020; 39: 172-180.
26. Kanellopoulos AJ. Comparison of sequential vs same-day simultaneous collagen cross-linking and topography-guided PRK for treatmentof keratoconus. J Refract Surg 2009; 25: S812-S818.
27. Alfonso JF, Fernandez-Vega L, Lisa C, et al. Collagen copolymer toric posterior chamber phakic intraocular lens in eyes with keratoconus. J CatarRefract Surg 2010; 36: 906-916.
28. Greenstein SA, Fry KL, Bhatt J, et al. Natural history of corneal hazeafter collagen crosslinking for keratoconus and corneal ectasia: scheimpflug and biomicroscopic analysis. J Cataract Refract Surg 2010; 36: 2105-2114.
29. Lopes B, Ramos I, Ambrosio R Jr. Corneal densitometry in keratoconus. Cornea 2014; 33: 1282-1286.
30. Ni Dhubhghaill S, Rozema JJ, Jongenelen S, et al. Normative values forcorneal densitometry analysis by Scheimpflug optical assessment. InvestOphthalmol Vis Sci 2014; 55: 162-168.
31. Elflein HM, Hofherr T, Berisha-Ramadani F, et al. Measuring cornealclouding in patients suffering from mucopolysaccharidosis with the pentacam densitometry programme. Br J Ophthalmol 2013; 97: 829-833.
32. Otri AM, Fares U, Al-Aqaba MA, et al. Corneal densitometry as anindicator of corneal health. Ophthalmology 2012; 119: 501-508.
33. Cennamo G, Forte R, Aufiero B, et al. Computerized scheimpflug densitometry as a measure of corneal optical density after excimer laserrefractive surgery in myopic eyes. J Cataract Refract Surg 2011; 37: 1502-1506.
34. Wollensak G, Spoerl E, Wilsch M, et al. Keratocyte apoptosisafter corneal collagen cross-linking using riboflavin/UVA treatment. Cornea 2004; 23: 43-49
35. Wilson SE, Kim W-J. Keratocyte apoptosis: implications oncorneal wound healing, tissue organization, and disease. Invest Ophthalmol Vis Sci 1998; 39: 220-226.
36. Alzahrani K, Mofty H, Lin EY, et al. Corneal Imaging and Densitometry Measurements in Juvenile and Adult Keratoconus Patients to Evaluate Disease Progression and Treatment Effects After Corneal Cross-Linking. Clin Optom (Auckl) 2019; 11: 173-180.
37. Böhm M, Shajari M, Remy M, et al. Corneal densitometry after accelerated corneal collagen cross-linking in progressive keratoconus. Int Ophthalmol 2019; 39: 765-775.
38. Shen Y, Jian W, Sun L, et al. One-Year Follow-Up of Changes in Corneal Densitometry After Accelerated (45 mW/cm^2) Transepithelial Corneal Collagen Cross-Linking for Keratoconus: A Retrospective Study. Cornea 2016; 35: 1434-1440.
39. Badawi AE. Corneal endothelial changes after accelerated corneal collagen cross-linking in keratoconus and postLASIK ectasia. Clin Ophthalmol 2016; 10: 1891-1898.
40. Cingü AK, Sogutlu-Sari E, Cınar Y, et al. Transient corneal endothelial changes following accelerated collagen cross-linking for the treatment of progressive keratoconus. Cutan Ocul Toxicol 2014; 33: 127-131.