Epithelium-off versus epithelium-on corneal collagen cross-linking with accelerated UV – a protocol for treatment of keratoconus

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Purpose
Our purpose was to compare the efficacy of 'epithelium-off' and 'epithelium-on' cross-linking (CXL) in treatment of progressive keratoconus.
Patients and methods
This study included 48 eyes of 26 patients who met our inclusion criteria. The Epi-Off CXL group included 32 eyes of 17 patients, and the Epi-On CXL group included 16 eyes of nine patients. Preoperative assessments of uncorrected and best-corrected visual acuities, refractive errors, keratometry, and corneal tomography including pachymetry, were compared with the postoperative values.
Results
Preoperatively, there was a statistically nonsignificant difference between the two groups in all studied variables except for the pachymetry at thinnest location. In the Epi-Off group, there was a significant improvement of uncorrected visual acuity, best-corrected visual acuity, K_{max}, and inferior–superior value at the 12-month visit. There was late significant worsening of the back elevation and spherical equivalent at the 12-month visit and also significant thinning of pachymetry at thinnest location associated with significant worsening of the average thickness increase. All other variables showed nonsignificant change (stabilization) at both postoperative visits. In the Epi-On group, there was significant thinning of pachymetry at thinnest location and stabilization of uncorrected corrected visual acuity, best-corrected visual acuity, K_1, K_{max}, (inferior–superior), *Y*-coordinate, and front elevation at both postoperative visits, and early stabilization with late worsening of all of other variables.
Conclusion
The Epi-Off CXL was found to be more superior to Epi-On CXL in terms of stabilization of progressive keratoconus but was inevitably associated with complications related to epithelial debridement.

Keywords:
cross-linking, Epi-Off cross-linking, Epi-On cross-linking, keratoconus

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Introduction

Keratoconus (KC) is a degenerative corneal disease characterized by being a progressive, noninflammatory, bilateral, but asymmetrical disorder that affects the stromal biomechanical stability resulting in forward protrusion of the cornea [1,2]. KC has a very high effect on the quality of life of patients, owing to either the disease itself or the treatments that were available before the advent of corneal collagen cross-linking (CXL) [3].

CXL is the first treatment that targets the basis of progression of KC, the biomechanical weakness, through stiffening the cornea and arresting the progression of KC [4].

The standard procedure of CXL, described in 2003 [4], involved the removal of central 8–9 mm of corneal epithelium to allow the passage of the hydrophilic macromolecule of riboflavin into the stroma [5]. However, corneal de-epithelialization was found to be related to some postoperative complications such as postoperative pain, temporary visual diminution, healing problems of the epithelium, anterior stromal haze, corneal infection, herpes virus reactivation, and even corneal melting [6,7].

Leaving the epithelium intact, during the CXL procedure, has the advantage of avoiding the aforementioned postoperative complications related to epithelial removal [8]. Moreover, thinner corneas, which are only 400 μm (with epithelium), may be safer to be treated by the Epi-On rather than the Epi-Off

CXL, as the endothelium is better protected by Ultrviolet-A (UVA)-filtering effect of the intact epithelium [9]. However, leaving the epithelium intact has been related to particular drawbacks that can decrease the efficacy of Epi-On CXL; these drawbacks include preventing riboflavin penetration and homogenous saturation of the stroma [10], reducing oxygen diffusion into the stroma and, on the contrary, it consumes 10 times more oxygen than stromal layer of comparable thickness [11,12], and finally, blocking the UVA penetration into the stroma [13].

Thus, Epi-On CXL mandates a special type of riboflavin that can penetrate through intact epithelium [8]. It should contain enhancers such as EDTA, benzalkonium chloride (BAC), and trometamol and the topical anesthetic tetracaine 1% which is reported to loosen epithelial tight junctions and facilitate epithelial permeability [14–16]. Despite this, the epithelium is still a major barrier for riboflavin penetration even with the prolonged application of a cationic surfactant such as BAC [12].

The standard procedure is time consuming and troublesome for both the patient and the surgeon. The reduction of CXL procedure duration can be achieved through either a shorter riboflavin administration time with improved corneal penetration, e.g. iontophoresis, or through the application of higher ultraviolet ray (UV) doses [17]. The theoretical background of the latter modification is based on the photochemical reciprocity law (Bunsen–Roscoe law), which states that, the effect of a photochemical or photobiological reaction is directly proportional to the total irradiation dose, irrespective of the time span over which the dose is delivered [18,19].

Patients and methods

Study design

A nonrandomized, noncontrol, comparative interventional prospective study was conducted.

Patient population

This study included 48 eyes of 26 patients, who met our inclusion criteria and were of stage I–II according to Amsler–Krumeich classification [20,21]. The study was carried out from July 2014 to December 2016. The patients were divided into two groups: the Epi-Off CXL group that underwent epithelial removal before riboflavin instillation (32 eyes of 17 patients) and the Epi-On CXL group, where epithelium was not removed (16 eyes of nine patients). This division was not randomized and depended mainly on the corneal thickness if it allowed or not the epithelial removal with a minimum residual corneal thickness of 400 μm. In both groups, the accelerated UVA treatment protocol was used.

The study was approved by the Institutional Review Board/Ethics Committee of the Faculty of Medicine at Assiut University and was conducted in accordance with the Declaration of Helsinki. Every patient was informed about his or her condition, the nature of the procedure, and its possible consequences, and a written consent was obtained from each patient or from the parents, if the patient was younger than 18 years.

The study was carried out in three private eye centers (Alnoor, Teba, and Alforsan centers) in Assiut where the equipments are available, after approval from administration of each center.

Inclusion criteria

The following inclusion criteria were applied:

(1) Progressive KC with a maximum corneal power (K_{max}) less than 60 D.
Progressive KC was identified when one or more of the following characteristics were found during a period of 6–12 months before treatment: loss of two or more lines of the corrected distant visual acuity on Snellen chart in 1 year [22], an increase in the cylinder magnitude on manifest refraction by greater than or equal to 1.00 D in 1 year [23], an increase in the manifest refraction spherical equivalent (MRSE) by greater than or equal to 1.00 D in 1 year [22], an increase in the mean K (K_m) [24] or maximum K (K_{max}) by greater than or equal to 1.00 D in 1 year [25], or a decrease in central corneal thickness by more than or equal to 5% in 6 months [6].
(2) Corneal thickness without epithelium greater than or equal to 400 μm (in the Epi-Off CXL group). The cornea was eligible for Epi-On CXL if the corneal thickness with epithelium greater than or equal to 400 μm.
(3) Age of patient between 14 and 40 years.

Exclusion criteria

The following exclusion criteria were applied:

(1) Corneal scarring.
(2) Epithelial healing disorders, e.g.:
    (a) Recurrent corneal erosion syndrome or.
    (b) History of diseases that may delay corneal healing or predispose the eye to future complications (e.g. rheumatic disorders,

glaucoma, uveitis, chemical burn, and corneal dystrophy).
(3) History suggestive of herpetic keratitis because the UVR can activate herpes virus.
(4) History of previous corneal surgery or iatrogenic ectasia.
(5) Pregnancy and breast-feeding.

Preoperative evaluation included full history taking and ophthalmological examination in addition to topographical evaluation using the Pentacam Comprehensive Eye Scanner (Oculus Optikgera, Wetzlar, Germany).

Early postoperative follow-up

Follow-up was aimed at detecting and treating any postoperative complication. It was done at the slit-lamp on the first, third, and sixth postoperative days, and then at 2 weeks, 1 month, and 3 months.

Late postoperative follow-up

Follow-up of the patient at the sixth and 12th month postoperatively was aimed at evaluation of CXL visual, refractive, and topographical effects.

Surgical technique

The UVA-emitting device used in the study was Vega CBM X-Linker (CSO, Italy) which emits at 370 nm to produce 10 mW/cm^2, which when used for 9 min produces a total energy of 5.4 J/cm^2.

Before treatment, the irradiance of the UV machine was calibrated using a UV light meter (Baush and Lomb, New York, USA) (YK-35UV). The radiant energy was acceptable when it was ±10% of the intended energy.

Patient preparation was carried out before the patient was brought to the operative room through administration of pilocarpine hydrochloride 2% (ocucarpine 2%; Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) miotic eye drops every 10 min for three times to reduce the risk of UV exposure of retroiridal eye structures, prophylactic antibiotic drop of moxifloxacin hydrochloride 0.5% (Vigamox; Alcon, Fort Worth, Texas, United States) every 5 min for four times, and one drop of the topical anesthetic, benoxinate hydrochloride 0.4% (Benox; E.I.P.I. Co., Cairo, Egypt) every 5 min for four times.

The skin around the eyes was wiped with 10% povidone–iodine solution (Betadine 10%; El-Nile Co., Cairo, Egypt) and a sterile draping was applied. Another drop of topical anesthesia was instilled before the insertion of lid speculum.

Regarding the epithelium for the Epi-Off CXL group, the central 8–9 mm of corneal epithelium was marked with a caliber and removed by mechanical debridement using a blunt Hockey Stick Knife (Huaian Tisurg Medical Instruments Co.). For the Epi-On CXL group, the epithelium was not removed, but instead, its permeability to riboflavin was enhanced by the instillation of topical anesthetic eye drops in addition to the BAC preservative (0.01%) present in the eye drops as well as in the transepithelial riboflavin solution.

Regarding riboflavin instillation, before starting the riboflavin instillation, the room lights were decreased to avoid affecting the composition and efficacy of riboflavin, and also the syringe that contains the riboflavin was covered by a sterile towel to avoid exposure to light. Riboflavin must have been kept in the refrigerator at +4 to +8°C. For the Epi-off CXL group, MedioCROSS-M (Medio-Haus-Medizin Produkte GmbH) was used, whereas for the Epi-On CXL group, MedioCROSS-TE (Medio-Haus-Medizin Produkte GmbH) was used. Instillation of either type continued every 2 min for 30 min. At the end of the 30 min, stromal absorption of riboflavin was confirmed under the surgical microscopic of WaveLight Allegretto 200 Hz laser (Alcon Laboratories Inc.).

The UVA radiation was focused on the central 8 mm of corneal surface at the wavelength of 370 nm to give a total dose of 5.4 J/cm^2 through the accelerated protocol (10 mW/cm^2 for 9 min).

During the UVA treatment, protection of the surgeon eyes was done using protective goggles that block the wavelength of 370 nm (Ellex, Australia) in addition to the usual personal protective equipments including the gown, overhead, facemask and gloves; protection of the limbal area by accurate focusing; and meticulous centration of the UV circle. Riboflavin instillation was continued every 3 min during UVA treatment, and topical anesthesia was instilled whenever the patient complained from pain or burning sensation.

After UVA treatment was finished, washing of riboflavin solution from the corneal surface and conjunctival sac was done using balanced salt solution. Then, topical broad-spectrum antibiotic drops, e.g. moxifloxacin (Vigamox; Alcon), were instilled and a bandage contact lens (Bausch & Lomb PureVision; Baush and Lomb, New York, USA) was then fitted onto the cornea in both of Epi-Off and Epi-On CXL groups.

Postoperative treatment included preservative-free Moxifloxacin (Vigamox; Alcon) five times per day.

Steroid/antibiotic combination eye drops, tobramycin 0.3% plus dexamethasone 0.1% (TobraDex; Alcon), were instilled twice per day until re-epithelization and removal of contact lens, then it was increased to five times per day for a week, and then two times per day for another 2 weeks in the Epi-Off group, whereas for the Epi-On group, it was given five times per day from the second postoperative day. Preservative-free tears substitute, carboxymethylcellulose sodium 0.5% (Refresh Plus; Allergan, Dublin, Republic of Ireland), five times per day, was used for 3–4 weeks. Oral analgesic was prescribed three times per day to relief pain until re-epithelialization, e.g. ibuprofene (Brufen; Abbott) 400 mg three times per day for adults or 200 mg three times per day for children below 18 years. The patient was advised to use sunglasses for 2 weeks.

Statistical analysis

Statistical analysis was done using the 'statistical package for the social sciences' (version 16.0; SPSS Inc., Chicago, Illinois, USA) for analysis. The uncorrected and best-corrected visual acuities (UCVA and BCVA) were measured in decimal notation, which was converted to logMAR notation because it is more suitable for statistical analysis, whereas the decimal notation was used for descriptive purposes because it is easier to understand than the logMAR.

The normality of data was checked using Kolmogorov–Smirnov (K-S) test which found that the data of each group were not normally distributed. Preoperative and postoperative parameters within each group were compared using the nonparametric Wilcoxon's signed rank test. Postoperative parameters were compared between the two groups using the nonparametric Mann–Whitney test. *P* value of less than 0.05 was considered to be statistically significant.

Results

Our study included 48 eyes of 26 patients, 12 (46%) males and 14 (54%) females, whose age ranged from 14 to 38 years, with a mean age of 23.80±7.10 years. Those 48 eyes were divided into two groups: the Epi-Off CXL group included 32 eyes of 17 patients, and the Epi-On CXL group included 16 eyes of nine patients.

There was no statistically significant difference between the two groups at preoperative baseline except for the 'Pachymetry at thinnest location' that showed statistically significant difference, which was expected as it was much thinner in the Epi-On, see Table 1.

The preoperative baseline and the postoperative mean values for each variable in both groups are presented in Table 2. The significance of change from the preoperative mean value to the 6-month mean value was represented by P_1, whereas the significance of change from the preoperative mean value to the 12-month mean value was represented by P_2. The amount of change from the preoperative value to the 6-month value was compared between the two groups, Epi-Off and Epi-On groups; the significance of difference between both groups at 6 months was described by P_3; and the difference between the two groups at 12 months was described by P_4. Any *P* value of these four was considered statistically significant when it was less than or equal to 0.05. For P_1 and P_2 values, * indicates significant improvement and # indicates significant worsening. For P_3 and P_4 values, § indicates significant difference between the two groups.

The UCVA and BCVA significantly improved in the Epi-Off group but showed nonsignificant change with the Epi-On group, so the Epi-Off group had significantly better visual outcome than the Epi-On group at the 12-month follow-up, but at the 6-month follow-up, there was no significant difference.

The MRSE, the refractive cylinder, and the corneal astigmatism, all showed significant worsening at the

Table 1 Preoperative mean and SD of each of the studied variables

Preoperative variables	Epi-Off CXL group	Epi-On CXL group	*P* value
Age	23.84±7.15	23.69±7.18	0.835
UCVA logMAR	0.89±0.4	0.87±0.38	0.832
BCVA logMAR	0.29±0.18	0.28±0.22	0.850
MRSE	−4.28±3.5	−4.92±3.4	0.505
Cylinder	−3.27±1.64	−4.62±3.0	0.145
Corneal astigmatism	−3.12±1.53	−3.88±2.24	0.314
K_1	45.39±3.0	44.86±2.52	0.801
K_2	48.5±3.44	48.74±3.18	0.694
K_m	46.88±3.10	46.67±2.61	0.965
K_{max}	51.97±4.8	53.06±4.99	0.638
I-S	5.26±3.87	8.26±5.44	0.073
Pachymetry at thinnest location	480.9±34.96	439.7±16.4	<**0.001**
Y-coordinate	−0.39±0.46	−0.61±0.44	0.279
Q-value	−0.73±0.39	−0.69±0.57	0.533
Front elevation	12.8±13.7	17.06±9.65	0.405
Back elevation	31.6±23.8	41.5±23.2	0.225
Average thickness increase	1.76±0.53	1.9±0.6	0.431

BCVA, best-corrected visual acuity; CXL, cross-linking; MRSE, manifest refraction spherical equivalent; UCVA, uncorrected corrected visual acuity; The significance of difference between the two groups is shown as *P* value, which was considered statistically significant when it was less than 0.05. Bold values: statistically significant difference.

Table 2 Preoperative and postoperative values of the studied variables

Parameters	CXL	Preoperative	6 months	12 months	P_1 value	P_2 value
UCVA (logMAR)	Epi-Off	0.89±0.40	0.80±0.44	0.75±0.43	0.008*	<0.001*
	Epi-On	0.87±0.38	0.77±0.37	0.85±0.42	0.138	0.798
		P_3=0.537	P_4=0.030§			
BCVA (logMAR)	Epi-Off	0.29±0.18	0.23±0.17	0.21±0.16	0.006*	<0.001*
	Epi-On	0.28±0.22	0.24±0.16	0.30±0.19	0.223	0.564
		P_3=0.265	P_4=0.002§			
MRSE	Epi-Off	–4.28±3.5	–4.32±3.3	–4.52±3.3	0.149	0.034#
	Epi-On	–4.92±3.4	–5.32±3.5	–6.06±3.5	0.069	0.003#
		P_3=0.430	P_4=0.005§			
Refractive cylinder	Epi-Off	–3.27±1.64	–3.16±1.7	–3.00±1.5	0.237	0.236
	Epi-On	–4.62±3.0	–4.58±2.9	–5.12±3.3	0.208	0.016#
		P_3=0.048§	P_4<0.001§			
Corneal astigmatism	Epi-Off	–3.12±1.53	–3.13±1.8	–3.15±1.6	0.740	0.613
	Epi-On	–3.88±2.24	–4.06±2.25	–4.28±2.23	0.170	0.004#
		P_3=0.392	P_4=0.038§			
K_1	Epi-Off	45.39±3.0	45.26±2.95	45.22±3.0	0.326	0.089
	Epi-On	44.86±2.52	44.94±2.62	45.04±2.7	0.669	0.348
		P_3=0.313	P_4=0.065			
K_2	Epi-Off	48.5±3.44	48.38±3.47	48.37±3.48	0.251	0.321
	Epi-On	48.74±3.18	48.99±3.33	49.32±3.38	0.089	0.011#
		P_3=0.055	P_4=0.002§			
K_m	Epi-Off	46.88±3.10	46.75±3.10	46.75±3.16	0.303	0.181
	Epi-On	46.67±2.61	46.86±2.77	47.14±2.8	0.195	0.026#
		P_3=0.102	P_4=0.003§			
K_{max}	Epi-Off	51.97±4.8	51.47±4.63	51.44±4.64	0.017*	0.009*
	Epi-On	53.06±4.99	53.11±4.47	53.49±4.47	0.660	0.196
		P_3=0.112	P_4=0.018§			
I-S	Epi-Off	5.26±3.87	4.73±3.26	4.57±3.32	0.095	0.015*
	Epi-On	8.26±5.44	7.69±4.44	7.91±4.33	0.254	0.605
		P_3=0.974	P_4=0.341			
Thinnest location	Epi-Off	480.9±34.96	470.9±38.16	470.3±36.74	<0.001#	0.001#
	Epi-On	439.7±16.4	432.6±14.6	424.8±15.7	0.017#	0.001#
		P_3=0.622	P_4=0.134			
Y-coordinate	Epi-Off	–0.39±0.46	–0.33±0.49	–0.39±0.46	0.510	0.940
	Epi-On	–0.61±0.44	–0.54±0.34	–0.63±0.32	0.378	0.569
		P_3=0.615	P_4=0.511			
Q-value	Epi-Off	–0.73±0.39	–0.72±0.36	–0.77±0.36	0.806	0.308
	Epi-On	–0.69±0.57	–0.76±0.52	–0.84±0.55	0.062	0.004#
		P_3=0.125	P_4=0.039§			
Front elevation	Epi-Off	12.8±13.7	12.25±13	12.81±12.9	0.218	0.600
	Epi-On	17.06±9.65	16.19±9.7	18.81±9.9	0.384	0.122
		P_3=0.973	P_4=0.094			
Back elevation	Epi-Off	31.6±23.8	34.4±22.3	35.8±22.6	0.054	0.001#
	Epi-On	41.5±23.2	41.19±25.3	47.06±24.5	0.796	0.021#
		P_3=0.424	P_4=0.224			
Average thickness increase	Epi-Off	1.76±0.53	1.9±0.59	1.9±0.59	0.001#	<0.001#
	Epi-On	1.9±0.6	1.9±0.67	2.03±0.66	0.453	0.014#
		P_3=0.080	P_4=0.870			

BCVA, best-corrected visual acuity; I-S, inferior–superior at the 5-mm circle of sagittal curvature map; logMAR, logarithm of minimum angle of resolution; MRSE, manifest refraction spherical equivalent; UCVA, uncorrected visual acuity; *Y*-coordinate, vertical displacement of thinnest location. *Indicates significant improvement and #Indicates significant worsening. For P3 and P4 values, §Indicates significant difference between the two groups.

12-month follow-up in the Epi-On group, but only the MRSE showed significant worsening at the 12-month visit in the Epi-Off group, which showed significantly better outcome of MRSE, refractive cylinder, and corneal astigmatism than the Epi-On group at the 12-month follow-up and for refractive cylinder at the 6-month follow-up.

The K_1 showed nonsignificant change at both postoperative visits for both groups and there was no

significant difference when comparing the change of K_1 at 6 and 12 months for the two groups.

For the K_2, K_m, and Q-value, the Epi-Off group showed nonsignificant change at both postoperative visits, whereas the Epi-On group showed significant worsening at the 12-month follow-up. When comparing the two groups, the Epi-Off group resulted in significantly better outcome at the 12-month visit.

The K_{max} showed significant improvement at both postoperative visits with the Epi-Off group, unlike the Epi-On group which showed nonsignificant change. On comparing the two groups, the Epi-Off group was significantly better at the 12-month visit.

There was significant improvement of the inferior–superior (I-S) value at the 12-month visit in the Epi-Off group, whereas there was nonsignificant change at both visits in the Epi-On group, but there was nonsignificant difference between the two groups.

Pachymetry at the thinnest location showed significant thinning at both visits in the two groups; moreover, there was nonsignificant difference between the two groups.

The vertical displacement of the thinnest location (Y-coordinate) and the front elevation showed nonsignificant change at both visits in both groups, and there was nonsignificant difference between the two groups.

The back elevation showed significant worsening at the 12-month visit in both groups, and there was nonsignificant difference between the groups.

The average thickness increase showed significant worsening at both postoperative visits in the Epi-Off group, whereas the Epi-On group showed significant worsening of the average thickness increase at the 12-month visit. Despite that, there was nonsignificant difference between the two groups.

Postoperative complications

Delayed re-epithelialization

Delayed re-epithelialization beyond 5 days postoperatively was found in four (12.5%) eyes of the 32 eyes that underwent Epi-Off CXL. Three of the four eyes had spring catarrh with one eye had steroid-induced cataract, whereas the fourth eye was not associated with any history of systemic diseases. All of the four eyes that had delayed re-epithelialization have recovered, and the corneal abraded area was re-epithelialized by the 12th day, but all left behind an anterior stromal haze.

In the Epi-On group, immediate postoperative slit-lamp examination revealed epithelial edema and punctate epitheliopathy in all eyes, but no corneal abrasions were found. These findings disappeared completely by the third postoperative day.

Anterior stromal haze

It was the most common complication noticed during the postoperative period as it was seen in 19 of 48 (39.6%) eyes (Table 3).

All of the eyes that developed anterior stromal haze had a mild degree of clouding that was of grade 1 according to the scoring system modified by Greenstein *et al.* [26].

Anterior stromal haze decreased in density during the follow-up period of 1 year. The eye that developed stromal haze in the Epi-On group showed complete resolution by the third month. For the 18 eyes of the Epi-Off group, 14 eyes showed complete resolution of the haze by the sixth month, whereas in the remaining four eyes, which were associated with delayed re-epithelialization, the haze disappeared at the 12-month follow-up.

Treatment failure

For the whole study population, in nine of 48 (18.7%) eyes, the CXL failed to prevent progression of KC (Table 4). Failure was identified by one of the criteria suggested by Shalchi *et al.* [27] and Poli *et al.* [28].

Discussion

Visual acuity (UCVA and BCVA) showed significant improvement at both postoperative visits in the Epi-Off group, which was similar to Badawi [29] and Chow *et al.* [30]. However, this was unlike the results of Waszczykowska and Jurowski [17], Elbaz *et al.* [31], and Sadoughi *et al.* [22], who reported nonsignificant change of vision that can be attributed to their use of different riboflavin solution containing 20% dextran and doubtful corneal saturation because of not using lid speculum allowing the lid to blink and sweep the

Table 3 Number and percentage of eyes developed anterior stromal haze

	Anterior stromal haze	Number of stromal haze (% of eyes with haze)
Epi-Off	18	14 (56.3)
Epi-On	1	15 (6.25)

Table 4 Success and failure rates of cross-linking in each group of treatment

Types of surgery	Outcomes	Number of eyes (%)
Epi-Off CXL	Success	29 (90.6)
	Failure	3 (9.4)
	Total	32 (100)
Epi-On CXL	Success	10 (62.5)
	Failure	6 (37.5)
	Total	16 (100)

CXL, cross-linking.

riboflavin solution off the cornea [17], or instillation of riboflavin every 5 min rather than every 2 min [22]. Moreover, Helena *et al* stated usage of 50% alcohol in epithelial debridement [31] which can lead to substantial damage to the underlying stroma causing more keratocyte loss and more corneal edema and haze [32].

Dextran can lead to stromal dehydration resulting in intraoperative corneal thinning [33]. Moreover, dextran was found to inhibit the paracellular transport of riboflavin [34]. Replacing dextran with hydroxypropyl methylcellulose not only avoids the thinning effect of dextran and but also can increase the corneal thickness during CXL [35,36].

The UCVA and BCVA in our Epi-On were stabilized with statistically nonsignificant change at both postoperative visits, which was similar to the results of Gatzioufas *et al*. [11]. However, Zhang *et al*. [37] reported statistically significant improvement of UCVA at both postoperative visits but nonsignificant improvement of the BCVA at both postoperative visits.

The maximum keratometry (K_{max}) showed significant improvement at both postoperative visits with the Epi-Off group, which is similar to the results reported by Badawi [29], and Ozgurhan *et al*. [38], but unlike the results reported by Elbaz *et al*. [31] and Sadoughi *et al*. [22], who found stabilization of K_{max} with no significant change, which may also be attributed to their use of different riboflavin solution containing 20% dextran. The Epi-On group shown nonsignificant change of K_{max} at both postoperative visits, which was consistent with the results of Gatzioufas *et al*. [11] and Zhang *et al*. [37].

The pachymetry at the thinnest location showed significant thinning at both postoperative visits in both groups which was consistent with many studies [11,17,22,29,30]. On the contrary, Ozgurhan *et al*. [38] found nonsignificant change of the central corneal thickness at both postoperative visits, which may be because they used a different machine that combines Placido and Scheimpflug technologies, because some authors claim that the Scheimpflug-derived K_{max} and pachymetry can deteriorate whereas the Placido disc-derived features appear stable [14]. However, it was suggested that there is no 'real' reduction in corneal thickness after CXL [39], and that CXL modifies the optical density of the corneal stroma, influencing all pachymetric systems to different degrees [40]. This can explain why Zhang *et al*. [37] found nonsignificant change of the thinnest location at both of postoperative visits, as they did not detect any demarcation line through anterior segment optical coherence tomography which means that the optical density of the corneal stroma did not change, thus measuring with Pentacam will not give significant thinning [39,40].

The MRSE and the back elevation showed nonsignificant change at the 6-month visit and significant worsening at the 12-month visit in both groups. Same result was reported by Chan *et al*. [41], for the back elevation in the Epi-Off group. On the contrary, most studies found nonsignificant change of both MRSE and back elevation at both postoperative visits in both groups [29,31,37,38].

All other 10 variables studied in the Epi-Off group showed nonsignificant change at both postoperative visits, which means stabilization of these variables, except for the I-S value, which showed significant improvement at the 12-month visit, and for the average thickness increase, which showed significant worsening at both postoperative visits, which may be a reflection of the significant thinning of the cornea at both postoperative visits.

In the Epi-On group, four of these 10 variables showed nonsignificant change at both postoperative visits, which means stabilization of these variables, which include K_1, I-S, *Y*-coordinate, and front elevation. The remaining six variables include the refractive cylinder, corneal astigmatism, K_2, K_m, *Q*-value, and average thickness increase. These six variables showed stabilization at the 6-month visit but significant worsening at the 12-month visit, which means stabilization effect has short duration at the first 6 months postoperatively. The published data about the efficacy of Epi-On CXL are generally disappointing, although there is general acceptance that it is a safe procedure [11,13,14,42,43].

Delayed re-epithelialization beyond 5 days was seen in 12.5% of eyes in the Epi-Off group, and this is consistent with the rate of 17.4% reported by

Wajnsztajn *et al*. [44]. Three out of the four eyes that had delayed re-epithelialization in our study gave history of spring catarrh which can explain the delay in epithelial healing, meanwhile, the fourth eye had no history of atopic or autoimmune diseases. Other possibilities that may have led to the delayed epithelial healing are either limbal stem cell injury caused by inadvertent exposure to UVA [45], because we did not use the silicone ring or Merocel shield ring, or the neurotoxic effect of the CXL [46].

This finding was a common finding in Epi-On studies. The immediate postoperative epithelial edema and punctate epitheliopathy seen in all cases of the Epi-On group disappeared by the third postoperative day. This is consistent with other Epi-On studies which reported epithelial changes that ranged from simple punctate epitheliopathy [6], to even frank epithelial defect, as reported by Gatzioufas *et al*. [11] in 46% of eyes.

The anterior stromal haze is because of keratocyte apoptosis and subsequent repopulation [47], leading to a clinically visible demarcation line [48]. Haze was seen in 56.3% of the eyes in the Epi-Off group, which is less than the rate reported by Sherif [49] who found anterior stromal haze in 71% of Epi-Off group. This can be explained by his use of sharp knife in epithelial removal that may have injured the Bowman's layer. In the Epi-On group, the haze was noted in one of 16 (6.25%) eyes. Keratocyte apoptosis occurs to a lesser extent after Epi-On CXL [50]; this may be the reason why haze is less in Epi-On CXL.

Treatment failure is defined as worsening of KC owing to continued progression, which is identified by one of the following criteria suggested by Shalchi *et al*. [27] and Poli *et al*. [28].

(1) An increase in maximum K (K_{max}) readings of 1.0 D over the preoperative value. K_{max} is arguably the most important parameter when considering KC progression, and hence, treatment failure [27].
(2) A decrease of more than 0.1 (one line) in logMAR uncorrected or BCVA [28].
(3) An increase of keratometric values (K_1, K_2, and K_m) by greater than 0.75 D [28].

Failure of CXL was found in three out of 32 eyes treated in Epi-Off group giving a rate of 9.4% which was consistent with the failure rate in previous work by Shetty *et al*. [51], who reported three eyes out of 30 (10%) eyes. Ng *et al*. [52] and Waszczykowska and Jurowski [17] reported failure rate of 8.3 and 6.25%, respectively. The lower failure rate in the last two studies may be because they excluded patients younger than 18 years. The younger age group below 18 years old has more aggressive disease [53,54], and was associated with decreased stabilization of the disease [13].

In the Epi-On group, failure rate was 37.5%, whereas Gatzioufas *et al*. [11] reported a failure rate of approximately 46%.

Summary

Treatment of KC with Epi-Off/accelerated CXL resulted in stabilization of almost all topographic parameters, which included K_1, K_2, K_m, corneal astigmatism, front elevation, anterior surface asphericity (Q-value), and the vertical displacement of thinnest location (Y-coordinate), and even resulted in significant improvement of the K_{max} and early stabilization followed by late significant improvement of the I-S value.

All of the aforementioned parameters have been stabilized or improved during the 1-year follow-up period. All of them, except the Y-coordinate, are related to the anterior corneal surface which means that the anterior corneal surface has been stabilized with some improvement in some of its parameters.

This was reflected on the visual acuity, both the UCVA and the BCVA, which revealed significant improvement, despite the late worsening of the MRSE and the nonsignificant change of refractive cylinder, as the improvement of visual acuity does not depend solely on the refractive error. The stabilization and relative improvement of the anterior surface parameters may have resulted in regularization of the anterior corneal surface, which may have led to improvement of higher order aberrations [38].

The back elevation was stabilized during the first follow-up but increased significantly at the end of the first postoperative year. The increase in back elevation together with thinning at the thinnest location both were reflected on the 'average thickness increase' which significantly increased at both postoperative visits.

Thus, the end result of Epi-Off CXL is stabilization with some improvement of the anterior surface-related parameters associated with continuation of worsening of the parameters related to the posterior surface. This means that the effect of Epi-Off/accelerated CXL is limited to the anterior cornea. This anteriorly located

effect stabilized the cornea during the first postoperative year but may require further follow-up.

Treatment of KC with the Epi-On/accelerated CXL resulted in stabilization of UCVA, BCVA, K_1, K_{max}, I-S, *Y*-coordinate, and front elevation.

It resulted in early stabilization with late worsening of most of the refractive and topographic parameters, which included MRSE, refractive cylinder, K_2, K_m, corneal astigmatism, *Q*-value, back elevation, and the average thickness increase.

So, this technique of treatment may have resulted in early (6-month) stabilization of all parameters except the pachymetry at the thinnest location. Six months later, there was significant worsening of half of the aforementioned parameters in addition to the pachymetry of the thinnest location.

Although the K_{max} and visual acuities were stabilized, K_2 and K_m got worsened. Thus, longer follow-up period is mandatory to know if the worsening will continue and extend to other parameters or not.

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Conflicts of interests
There are no conflicts of interest.

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