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Accelerated epithelium-on or accelerated epithelium-off corneal collagen cross-linking: Contralateral comparison study

Erdem Yuksel[1]*, Mehmet Ozgur Cubuk[2], Nuriye Gokcen Yalcin[3]

Abstract:

PURPOSE: The aim of the study is to compare the accelerated epithelial-on corneal collagen cross-linking (epi-on CXL) and accelerated epithelial-off corneal collagen cross-linking (epi-off CXL) in terms of clinical and confocal microscopy results.

MATERIALS AND METHODS: Forty-two eyes of 21 patients with progressive keratoconus and simultaneously undergoing accelerated epi-on CXL in one eye and accelerated epi-off CXL in other eye were evaluated. Uncorrected visual acuity (UCVA) and best-corrected visual acuity (BCVA) with spectacle in logMAR and topographic findings (mean keratometry [K_{mean}] and maximum keratometry [K_{max}]) were recorded at 1, 3, 6, 12, 18, 24, and 30 months. Eyes were compared in terms of subjective pain scores after the procedures. Furthermore, anterior segment optical coherence tomography and confocal microscopy were performed at 1 month.

RESULTS: K_{mean} and K_{max} were less than baseline in both the groups; however, the reduction was significantly higher in epi-off CXL than epi-on CXL eyes at 18 and 30 months. The UCVA and BCVA increased approximately 1 Snellen line at the end of mean follow-up in epi-off CXL and in epi-on CXL. Stromal demarcation line for epi-off CXL is 276.4 ± 58.9 while 148.3 ± 24.8 for epi-on CXL ($P = 0.001$). Furthermore, subepithelial nerves were observed in any eye in epi-off CXL; however, subepithelial nerves were observed in 12 eyes (80%), in epi-on CXL ($P = 0.01$).

CONCLUSION: Both techniques were able to stop progression; however, in contrast to expectations, the pain was felt more in epi-on CXL than epi-off CXL.

Keywords:

Epithelium-off corneal collagen cross-linking, keratoconus, transepithelial corneal collagen cross-linking

[1]Department of Ophthalmology, School of Medicine, Kastamonu University, Kastamonu, [2]Department of Ophthalmology, Istanbul Training and Research Hospital, Istanbul, [3]Department of Ophthalmology, Vezirkopru State Hospital, Samsun, Turkey

***Address for correspondence:**
Dr. Erdem Yuksel, Department of Ophthalmology, School of Medicine, Kastamonu University, Kuzeykent-Kastamonu, Turkey.
E-mail: rdmyksl@yahoo.com

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Introduction

Keratoconus is a progressive disease which is characterized by thinning, steepening, and conical shape of corneal tissue as a result of mechanical impairment of cornea, and corneal collagen cross-linking (CXL), which was described firstly by Wollensak *et al*., is an unique and effective method which hardens the cornea and stops or delays progression of the disease.[1] Conventional epithelial-off corneal cross-linking (epi-off CXL) requires that ultraviolet-A (UVA) is administered in 370 wavelength, with 3 mW/cm^2 power and 1 cm away from the cornea for 30 min following removal of epithelium (a total of 5.4 j/cm^2) and saturation of cornea with 0.1% riboflavin solution.[1] However, in recent years, accelerated epi-off CXL (aEpi-off CXL), in which the same amount of energy (5.4 j/cm^2) is given, has been described to shorten the time of treatment and with the same outcome as epi-off CXL

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was obtained with this technique.[2] Although epi-off CXL methods are effective, these methods cause severe pain and discomfort, temporary decreased visual acuity, stromal blurring, and risk of infections as a consequence of epithelium debridement.[3,4] Hence, to avoid these kinds of problems, epithelium-on corneal cross-linking (epi-on CXL), which does not require removal of epithelium, has been described.[5] Riboflavin is able to diffuse cornea easily after epithelium debridement; however, its hydrophilic and macromolecule properties limit the passage through the epithelium. Therefore, in epi-on CXL, substances impairing epithelial tight connections such as benzalkonium chloride (BAC), ethylenediaminetetraacetic acid (EDTA), and trometamol (TRIS) were added to riboflavin solution, and a new corneal cross-linking formula was achieved.[6] Riboflavin is able to pass through the epithelium and diffuses into the stroma owing to this new formula. However, many studies in the literature are showing conflicting findings about the effectiveness of epi-on CXL,[2,6-11] and there have not been any comparable studies about the effectiveness of both techniques which were performed simultaneously on the same patient. Therefore, in the present study, we aimed to compare accelerated epi-on CXL and epi-off CXL in terms of clinical and confocal microscopy results.

Materials and Methods

This comparative contralateral eye study was approved by the Local Ethics Committee of Istanbul Training and Research Hospital (no: 1396/17.03.2018). The tenets of the Declaration of Helsinki were followed in all steps of the study, and all patients were informed about its benefits and potential risks. Written informed consent was obtained from all the participants before the procedures.

Forty-two eyes of 21 patients (9 females and 12 males) with progressive keratoconus and simultaneously undergoing accelerated epi-on CXL in one eye and epi-off CXL in other eye were evaluated.

Epi-off CXL was performed on the right keratoconus eye, and epi-on CXL was performed on the left keratoconus eye.

Eyes were compared in terms of subjective pain scores (verbal rating scale[12]) after the procedures. Pain was scored ranging from 0 to 5.

Uncorrected visual acuity (UCVA) and best-corrected visual acuity (BCVA) with spectacle in logMAR and topographic findings (central corneal thickness (CCT), mean keratometry (K_{mean}), and maximum keratometry (K_{max}) were recorded at 1, 3, 6, 12, 18, 24, and 30 months. Furthermore, anterior segment optical coherence tomography (AS-OCT) (Heidelberg Spectralis spectral-domain OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) and confocal microscopy (*in vivo* confocal microscopy [IVCM]) (Confoscan 3.0, Nidek, Italy) were performed for corneal stromal demarcation line and presence of subepithelial nerves, respectively, at 1 month.

Keratoconus was diagnosed with a Scheimpflug camera and Placido disk-based topography (Sirius, CSO, Scandicci Firenze, Italy), and the same topographic device was used in the follow-up of the patients. The patients were classified according to the Amsler–Krumeich grading system.

The diagnosis of progressive keratoconus was based on an increase in maximum *K* (K_{max}) by higher than 1 D, the mean K (K_{mean}) by higher than 0.75 D, and corneal apex power by higher than 1 D in the past 12 months.

Excluded criteria were Stage 4 keratoconus diseases according to the modified Krumeich classification,[13] history of herpes virus keratitis, dry eye, corneal opacities, anomalies of the AS, and concurrent corneal infections.

Surgical technique

Accelerated epithelium-on corneal collagen cross-linking

0.5% proparacaine hydrochloride (Alcaine, Alcon Laboratories, Puurs, Belgium) and topical miotic drop 1% pilocarpine (Bilim Medicine, İstanbul, Turkey) were applied to each eye just before epi-on CXL. The transepithelial formulation of riboflavin (ParaCel™-VibeX Xtra™, Avedro Inc., Massachusetts, USA) was used for the procedure. ParaCel™ contains 0.25% riboflavin, hydroxypropyl methylcellulose (HPMC), benzalkonium chloride, ethylenediaminetetraacetate (EDTA), and TRIS, and VibeX Xtra™ contains 0.25% riboflavin and isotonic saline. First, ParaCel™ was administered every 1.5 min for 6 min, followed by administration of VibeX Xtra™ every 1.5 min for 6 min. After administration of these drugs for 12 min, penetration of riboflavin to the corneal stroma and anterior chamber was confirmed by slit-lamb examination. And then, accelerated corneal cross-linking was performed by exposing the central 9.0-mm cornea to UVA light at 365 nm wavelength (KXL System, Avedro Inc., Massachusetts, US) and with 9 mW/cm^2 power for 13 min (7.2 J/cm^2 energy). During UVA administration, isotonic solution was dropped every 2 min. Finally, topical 0.5% moxifloxacin (VIGAMOX®, Alcon Inc., Fort Worth, USA) was dropped, and therapeutic contact lens (Acuvue Oasys®, Johnson and Johnson Vision Care, Inc., USA) was placed.

Accelerated epithelium-off corneal collagen cross-linking

0.5% proparacaine hydrochloride (Alcaine, Alcon Laboratories, Puurs, Belgium) and topical miotic drop

1% pilocarpine (Bilim Medicine, İstanbul, Turkey) were applied to eye just before CXL. The central 9-mm corneal epithelium was debrided by crescent knife (Beaver®-Visitec International, Inc., Waltham, MA, USA) with the assisted 20% alcohol for epi-off CXL. Before UVA irradiation, as a photosensitizer, 0.1% riboflavin (VibeX™, Avedro Inc., Massachusetts, USA) CXL was applied. VibeX™ contains 0.1% riboflavin, dextran, and isotonic saline. VibeX™ ParaCel™ was administered every 3 min for 20 min as described using the manufacturers' suggested protocol. After administration of riboflavin for 20 min, penetration of riboflavin to the corneal stroma and anterior chamber was confirmed by slit-lamp examination. And then, accelerated corneal cross-linking was performed by exposing the central 9.0-mm cornea to UVA light at 365 nm wavelength (KXL System, Avedro Inc., Massachusetts, USA), with 9 mW/cm^2 power for 10 min (5.4 J/cm^2 energy). During UVA administration, VibeX™ was dropped every 3 min. Finally, topical 0.5% moxifloxacin (VIGAMOX®, Alcon Inc., Fort Worth, USA) was dropped, and therapeutic contact lens (Acuvue Oasys®, Johnson and Johnson Vision Care, Inc., USA) was placed.

After procedures, the patients received treatment of topical moxifloxacin four times daily for 1 week, unpreserved topical tear drops (Refresh®, Allergan Inc., Irvine, USA) every 2 h for 1 month, and fluorometholone acetate 0.1% (Flarex, Alcon Inc., Fort Worth, Texas, USA) three times daily for 1 month.

Statistical analysis

Statistical analysis for descriptive statistics was performed using 16.0 SPSS statistical software (IBM, New York, USA). Descriptive data were presented as the mean ± standard deviations. The normal distribution of the variables was tested by visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test). For intergroup (epi-on CXL and epi-off CXL) comparisons, the Mann–Whitney U-test was used; for intragroup (preoperative vs. postoperative data) comparisons, the Wilcoxon signed-rank tests were used. $P < 0.05$ was considered statistically significant.

Results

Forty-two eyes of 21 patients (9 females and 12 males) were included in the study. The mean age of the patients was 20.3 ± 4.6 years (range: 14–29 years). The demographic properties and the preoperative and postoperative mean values of patient parameters are summarized in Tables 1 and 2.

Subjective pain score

The mean pain score on the 1st day was 3.0 ± 0.57 in epi-off CXL and 3.7 ± 0.95 in epi-on CXL

Table 1: The baseline characteristics of patients (*n*=15)

	Epi-on CXL	Epi-off CXL	*P*†
Mean age (years), mean±SD	20.3±4.6	20.3±4.6	
Gender (female/male)	9/12	9/12	
The keratoconus stage*, mean±SD	2.7±0.6	2.6±0.5	0.57
UCVA (logMAR), mean±SD	0.7±0.3	0.7±0.1	0.52
BCVA (logMAR), mean±SD	0.5±0.3	0.4±0.2	0.25
Kmean (D), mean±SD	50.08±4.4	47.7±2.4	0.52
K_{max} (D), mean±SD	58.02±4.9	55.2±3.6	0.14
CCT (µm), mean±SD	435.5±36.8	453.2±21.7	0.16

*Amsler-Krumeich classification, †Mann-Whitney U-test, the preoperative comparison of Epi-on and Epi-off CXL. *n*=Number of patient, G=Gender, CCT (µ) (thinnest point)=Central corneal thickness, Kmean=Mean keratometry value, K_{max}=Maximum keratometry value, D=Diopter, UCVA=Uncorrected visual acuity, BCVA=Best-corrected visual acuity, SD=Standard deviation, logMAR=Logarithm of the minimum angle of resolution, CXL=Corneal cross-linking, Epi-on CXL=Epithelium-on CXL, Epi-off CXL=Epithelium-off CXL, CXL=Corneal collagen cross-linking

Table 2: The pre- and postoperative mean values of patient

	Epi-on CXL	Epi-off CXL	*P**
UCVA (logMAR), mean±SD			
Preoperative (*n*=15)	0.7±0.3	0.7±0.1	0.52
1 month (*n*=15)	0.7±0.3	0.7±0.2	0.51
3 months (*n*=15)	0.6±0.2	0.5±0.2	0.25
6 months (*n*=15)	0.7±0.2	0.6±0.3	0.24
12 months (*n*=15)	0.6±0.1	0.5±0.1	0.27
P†	0.27	0.08	
18 months (*n*=15)	0.6±0.1	0.5±0.2	0.57
P†	0.27	0.11	
24 months (*n*=5)	0.6±0.1	0.5±0.2	0.61
30 months (*n*=3)	0.6±0.1	0.5±0.2	0.63
BCVA (logMAR), mean±SD			
Preoperative (*n*=15)	0.5±0.3	0.4±0.2	0.25
1 month (*n*=15)	0.5±0.3	0.4±0.3	0.25
3 months (*n*=15)	0.4±0.2	0.3±0.2	0.40
6 months (*n*=15)	0.4±0.2	0.3±0.1	0.40
12 months (*n*=15)	0.4±0.2	0.2±0.1	0.35
P†	0.04	0.02	
18 months (*n*=15)	0.4±0.2	0.2±0.1	0.35
P†	0.04	0.01	
24 months (*n*=5)	0.4±0.1	0.2±005	0.38
30 months (*n*=3)	0.4±0.1	0.2±0.05	0.36
Kmean (D), mean±SD			
Preoperative (*n*=15)	50.0±4.4	47.7±2.4	0.52
1 month (*n*=15)	50.4±3.8	48.5±3.1	0.65
3 months (*n*=15)	50.4±3.9	48.1±3.0	0.61
6 months (*n*=15)	50.1±4.0	47.4±2.9	0.59
12 months (*n*=15)	49.5±3.4	46.9±2.7	0.04
P†	0.06	0.005	
18 months (*n*=15)	49.5±3.5	46.5±2.9	0.01
P†	0.06	0.005	
24 months (*n*=5)	49.6±3.1	46.4±2.8	0.03
30 months (*n*=3)	49.5±2.9	46.4±2.8	0.04
Kmean (D), mean±SD			
Preoperative (*n*=15)	58.0±4.9	55.2±3.6	0.14
1 month (*n*=15)	58.3±4.4	55.5±3.8	0.16
3 months (*n*=15)	57.8±4.1	54.8±4.1	0.10

Contd...

Table 2: Contd...

	Epi-on CXL	Epi-off CXL	*P**
6 months (*n*=15)	57.4±4.2	54.1±3.5	0.12
12 months (*n*=15)	57±4.5	54±3.4	0.13
P†	0.05	0.005	
18 months (*n*=15)	56.9±3.9	53.8±4.1	0.04
P†	0.05	0.005	
24 months (*n*=5)	57.2±4.2	54.1±4.8	0.32
30 months (*n*=3)	57.4±3.8	54.2±3.6	0.05
CCT, mean±SD			
Preoperative (*n*=15)	435±36.8	453±21.7	0.14
1 month (*n*=15)	428±45.2	423±44.1	0.05
P†	0.008	0.009	
3 months (*n*=15)	434±42.3	418±36.2	0.04
P†	0.85	0.007	
6 months (*n*=15)	435±39.2	416±28.3	0.04
P†	0.87	0.01	
12 months (*n*=15)	436±40.1	434±29.4	0.04
P†	0.51	0.02	
18 months (*n*=15)	434±37.3	439±33.2	0.02
P†	0.72	0.01	
24 months (*n*=5)	430±36.2	440±30.6	0.10
30 months (*n*=3)	430±37.2	441±31.8	0.16

*Mann-Whitney U-test, the comparison of Epi-on and Epi-off CXL in terms of reduction of Kmean, K_{max}, and CCT, in terms of increase of UCVA and BCVA. †Wilcoxon test, the comparison of pre- and postoperative UCVA, BCVA, Kmean, K_{max}, and CCT; at 12 months and at 18 months in Epi-on and Epi-off CXL. *n*=The number of patients at each follow-up, CCT (μ) (thinnest point)=Central corneal thickness, Kmean=Mean keratometry value, K_{max}=Maximum keratometry value, D=Diopter, UCVA=Uncorrected visual acuity, BCVA=Best-corrected visual acuity, SD=Standard deviation, logMAR=Logarithm of the minimum angle of resolution, CXL=Corneal cross-linking, Epi-on CXL=Epithelium-on CXL, Epi-off CXL=Epithelium-off CXL, CXL=Corneal collagen cross-linking

eyes (P = 0.004) [Figure 1]. There were no significant differences on the 2nd and 4th days between both the groups (P = 0.14 and P = 0.73, respectively).

Topographic findings

The preoperative K_{mean} and K_{max} were higher in epi-on CXL than epi-off CXL; however, the differences were statistically nonsignificant.

The preoperative K_{mean} was 47.7 ± 2.4 D and the postoperative K_{mean} was 46.9 ± 2.7 D and 46.5 ± 2.9 D at 12 and 18 months, respectively, in epi-off CXL (the reduction of K_{mean} at 12 months, P = 0.005, and at 18 months, P = 0.005). The preoperative K_{mean} was 50.08 ± 4.4 D and the postoperative K_{mean} was 49.5 ± 3.4 D and 49.5 ± 3.5 D at 12 and 18 months, respectively, in epi-on CXL (the reduction of K_{mean} at 12 months, P = 0.06, and at 18 months, P = 0.06).

The preoperative K_{max} was 55.2 ± 3.6 D and the postoperative K_{max} was 54 ± 3.4 D and 53.8 ± 4.1 D at 12 and 18 months, respectively, in epi-off CXL (the reduction of K_{mean} at 12 months, P = 0.005, and at 18 months, P = 0.005). The preoperative K_{max} was 58.0 ± 4.9 D and the postoperative K_{mean} was 57 ± 4.5 D

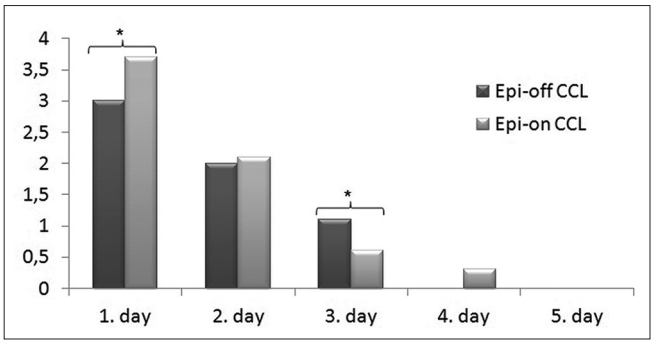

Figure 1: The mean pain score. *Mann–Whitney U-test, the comparison of epi-on and epi-off CXL, P = 0.042 on the 1st day and P = 0.044 on the 3rd day. CXL=Corneal collagen cross-linking

and 56.9 ± 3.9 D at 12 and 18 months, respectively, in epi-on CXL (the reduction of K_{mean} at 12 months, P = 0.05, and at 18 months, P = 0.05).

K_{mean} and K_{max} were significantly less than baseline at 12 and 18 months for epi-on CXL and epi-off CXL, and furthermore, the reduction of K_{mean} and K_{max} was significantly higher in epi-off CXL at 18 and 30 months (P = 0.01 and P = 0.04, P = 0.04 and P = 0.05 at 18 and 30 months, respectively) [Figure 2 and Table 2].

CCT was stable after epi-on CXL procedure; however, in epi-off CXL, CCT decreased during the first 6 months, and then, CCT started to increase, and at the end of 1st year, CCT reached preoperative values [Figure 3 and Table 2].

Uncorrected visual acuity and best-corrected visual acuity

In epi-on CXL group, the preoperative UCVA was 0.7 ± 0.3 and the postoperative UCVA was 0.7 ± 0.3, 0.6 ± 0.2, 0.7 ± 0.2, 0.6 ± 0.1, and 0.6 ± 0.1 at 1, 3, 6, 12, and 18 months, respectively; in epi-off group, the preoperative UCVA was 0.7 ± 0.1 and the postoperative UCVA was 0.7 ± 0.2, 0.5 ± 0.2, 0.6 ± 0.3, 0.5 ± 0.1, and 0.5 ± 0.2 at 1, 3, 6, 12, and 18 months, respectively.

In epi-on CXL group, the preoperative BCVA was 0.5 ± 0.3 and the postoperative BCVA was 0.5 ± 0.3, 0.4 ± 0.2, 0.4 ± 0.2, 0.4 ± 0.2, and 0.4 ± 0.2 at 1, 3, 6, 12, and 18 months, respectively.

In epi-off group, the preoperative BCVA was 0.4 ± 0.2 and the postoperative BCVA was 0.4 ± 0.3, 0.3 ± 0.2, 0.3 ± 0.1, 0.2 ± 0.1, and 0.2 ± 0.1 at 1, 3, 6, 12, and 18 months, respectively.

UCVA and BCVA increased approximately 1 Snellen line in epi-off CXL and in epi-on CXL (the increase of UCVA in epi-off CXL: P = 0.08 at 12 months and P = 0.11 at 18 months and in epi-on CXL: P = 0.27 at 12 months and P = 0.27 at 18 months; the increase of BCVA in epi-off CXL: P = 0.02 at 12 months and P = 0.01 at 18 months'

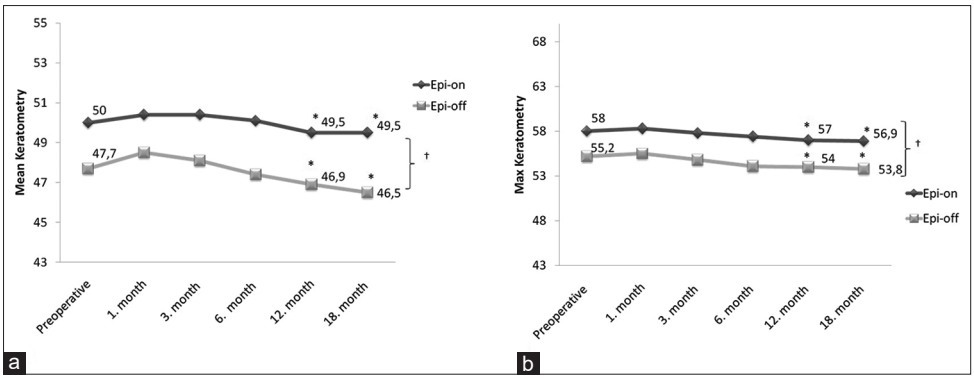

Figure 2: The measurements of keratometry before and after epi-on CXL and epi-off CXL. (a) The mean keratometry. *Wilcoxon signed-rank test, the reduction of K_{mean} in epi-off CXL: $P = 0.005$ at 12 months and $P = 0.005$ at 18 months' follow-up, and in epi-on CXL: $P = 0.06$ at 12 months and $P = 0.06$ at 18 months' follow-up. †Mann–Whitney U-test, the comparison of epi-on and epi-off CXL in terms of reduction of K_{mean} at 18 months' follow-up, $P = 0.011$. (b) The maximum keratometry. *Wilcoxon signed-rank test, the reduction of K_{max} in epi-off CXL: $P = 0.005$ at 12 months and $P = 0.005$ at 18 months' follow-up and in epi-on CXL: $P = 0.05$ at 12 months and $P = 0.05$ at 18 months' follow-up. †Mann–Whitney U-test, the comparison of epi-on and epi-off CXL in terms of reduction of K_{max} at 18 months, $P = 0.04$. CXL=Corneal collagen cross-linking

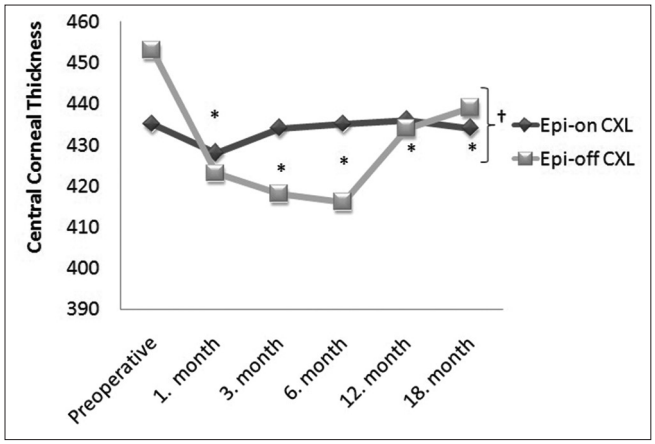

Figure 3: The measurement of central corneal thickness before and after epi-on CXL and epi-off CXL. *Wilcoxon test, the reduction of CCT in epi-off CXL: $P = 0.009$ at month 1, $P = 0.007$ at month 3, $P = 0.01$ at 6 months, $P = 0.02$ at 12 months, and $P = 0.01$ at 18 months and in epi-on CXL: $P = 0.008$ at month 1, $P = 0.85$ at month 3, $P = 0.87$ month 6, $P = 0.51$ at month 12, and $P = 0.72$ at 18 months. †Mann–Whitney U-test, the comparison of epi-on and epi-off CXL in terms of reduction in corneal thickness at 18 months, $P = 0.02$. CXL=Corneal collagen cross-linking

follow-up and in epi-on CXL: $P = 0.04$ at 12 months and $P = 0.04$ at 18 months). In addition, there was no significant difference between both the groups in terms of UCVA and BCVA improvement ($P = 0.57$ and $P = 0.68$, respectively) [Figure 4 and Table 2].

Anterior segment optical coherence tomography

Stromal demarcation line was observed in both the groups on the 1st month; however, demarcation line was more regular and deeper in epi-off CXL, while in epi-on CXL, demarcation line was irregular and shallower. The mean depth of stromal demarcation line was 276.4 ± 58.9 μm for epi-off CXL and 148.3 ± 24.8 μm for epi-on CXL ($P = 0.001$) at 1 month [Figure 5].

Confocal microscopy

Subepithelial nerves were not observed in any eye in epi-off CXL; however, in epi-on CXL, subepithelial nerves were observed in 12 eyes (80%) at 1 month ($P = 0.0001$). Furthermore, in epi-off CXL, IVCM disclosed intense apoptosis and haze in the anterior stroma, while mild apoptosis was observed in the anterior stroma in epi-on CXL [Figure 6].

Discussion

The progression of keratoconus can be effectively prevented by epi-off CXL;[1] however, removal of epithelium causes severe pain and discomfort, a transient decrease in visual acuity, problems of epithelial healing, stromal blurring, and risk of infections,[3,4] which has led to conduction of studies about corneal cross-linking without epithelial debridement. Corneal collagen cross-linking without epithelium debridement was first performed by Boxer Wachler *et al*. from the United States in 2004.[5] The riboflavin is a macro- and hydrophilic molecule that is not able to penetrate through the lipophilic corneal epithelium, so such substances in the formulation for epi-on CXL as BAC, EDTA, and TRIS disrupt epithelial tight connections and enable riboflavin to pass through the stroma. It is considered by many authors that the protection of epithelium during CXL reduces postoperative pain.[6-9,11] Magli *et al*. reported that pain scores were lower on the 1st day in the patients undergoing epi-on CXL than those undergoing epi-off CXL.[14] Besides the latter study, Stojanovic *et al*. observed no significant difference in the pain score.[11] However, in the present study, pain scores were higher in epi-on CXL eyes (6.0 in epi-off CXL and 7.8 in epi-on CXL) on the 1st day, and in addition, another study that compared pain scores between both techniques disclosed that epi-on CXL was more painful procedure.[15] It has been reported that epithelium debridement and resultant exposure of subepithelial nerves which lead to releases of prostaglandins and neuropeptides from these nerves cause more severe pain.[2,16] Al-Aqaba *et al*. and Touboul *et al*. showed in their confocal microscopy study that

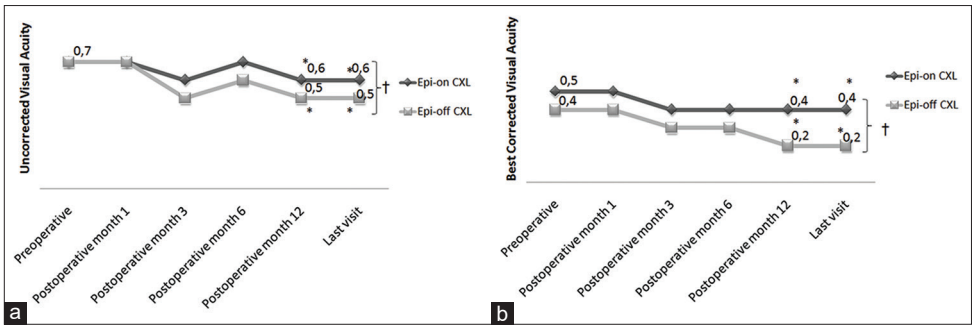

Figure 4: The visual acuity before and after epi-on CXL and epi-off CXL. (a) Uncorrected visual acuity. *Wilcoxon test, the increase of uncorrected visual acuity in epi-off CXL: $P = 0.08$ at 12 months and $P = 0.11$ at 18 months and in epi-on CXL: $P = 0.27$ at 12 months and $P = 0.27$ at 18 months. †Mann–Whitney U-test, the comparison of epi-on and epi-off CXL in terms of increase of uncorrected visual acuity, $P = 0.57$ at 18 months. (b) Best-corrected visual acuity with spectacle. *Wilcoxon test, the increase of best-corrected visual acuity in epi-off CXL: $P = 0.02$ at 12 months and $P = 0.01$ at 18 months' follow-up and in epi-on CXL: $P = 0.04$ at 12 months and $P = 0.04$ at 18 months. †Mann–Whitney U-test, the comparison of epi-on and epi-off CXL in terms of increase of best-corrected visual acuity, $P = 0.35$ at 18 months. CXL=Corneal collagen cross-linking

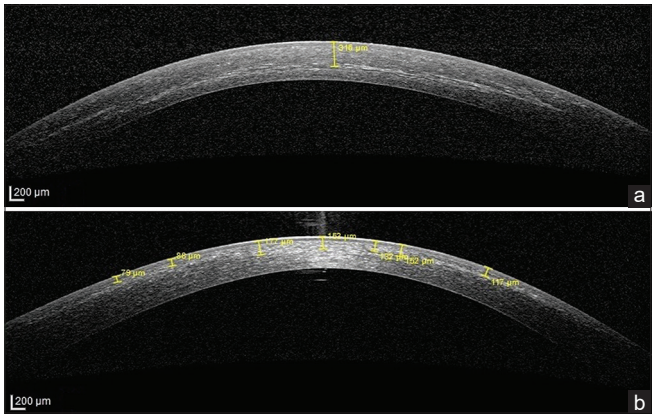

Figure 5: The stromal demarcation line on the 1st month. (a) Demarcation line in epi-off CXL, regular and deeper. (b) Demarcation line in epi-on CXL, irregular and shallower. CXL=Corneal collagen cross-linking

there were not any changes in subepithelial nerves after epi-on CXL; however, after epi-off CXL, subepithelial nerves were not seen.[2,17] And also, in the present study, there was a significant difference between epi-on CXL and epi-off CXL regarding the presence of subepithelial nerves. The presence of less severe pain after epi-off CXL and more severe pain after epi-on CXL suggests that subepithelial nerves are intact and are not exposed in the former procedure. Rather, they might be exposed in the latter one. Furthermore, another possible explanation for severe pain in epi-on CXL is cytokines which release from epithelium. These cytokines cause inflammation and pain consequently.

The major concern about epi-on CXL is the ability of stopping the progression of keratoconus. Many studies in the literature are showing conflicting findings about the effectiveness of epi-on CXL;[2,6-11,18-21] however, the formulation of riboflavin solutions that were used for each study was different. In our study, we used epi-on CXL riboflavin solution that was formulated by Avedro Inc. and consists of two steps – first: 0.25% riboflavin and HPMC, BAC, EDTA, and TRIS solution and then second: 0.25% riboflavin and isotonic saline solution are applied. At the end of mean follow-up, approximately 1 D reduction in K_{max} and K_{mean} was achieved in both techniques. We consider that the key point for the efficacy of transepithelial riboflavin solution is the presence of dextran and total UVA energy. Dextran is used for increasing the viscosity of iso-osmolar riboflavin solution; however, it was shown that the presence of dextran in transepithelial solution reduced the passage through the epithelium.[21,22] Koppen *et al*., Caporossi *et al*., Soeters *et al*., and Leccisotti and Islam[6-8,10,18] observed that transepithelial riboflavin solution which consisted of isotonic riboflavin with dextran and substances such as BAC, EDTA, and TRIS was not able to stop the progression of keratoconus. Another difference of riboflavin solution that we used is riboflavin concentration. The concentration of 0.25% may associate with the effectiveness of epi-on CXL. In addition, Stojanovic *et al*. compared the efficacy of epi-on CXL and epi-off CXL using hypotonic 0.5% riboflavin and showed that both methods were equally effective in stabilization of keratoconus. Further experimental investigations need for comparison of different riboflavin concentrations in transepithelial CXL.

It is well known that total UVA energy of 5.4 J/cm^2 is enough and nontoxic dose for epi-off CXL. However, total energy dose for epi-on CXL is controversial. Corneal epithelium and Bowman's layer decrease the passage of UVA. It was determined that the amount of blockage was approximately 20%–30%.[23-25] Hence, the total dose of UVA energy should increase, and 7.2 J/cm^2 dose is reasonable for epi-on CXL.

Another issue is demarcation line in CXL. Demarcation line is a transition zone from treated (acellular-apoptotic) anterior corneal stroma to untreated (cellular-nonapoptotic) posterior corneal stroma,[26,27] and the mean depth of demarcation line is approximately between 300 and 350 µm in standard epi-off CXL and

between 200 and 300 μm in accelerated epi-off CXL.[26,28-30] In the present study, the demarcation line in epi-off CXL was similar with these studies (276.4 ± 58.9). On the other side, in epi-on CXL, we found superficial demarcation that mean depth was 148.3 μm. Furthermore, Mastropasqua *et al*. demonstrated 106.61-μm demarcation depth in epi-on CXL. In addition, confocal microscopy confirmed AS-OCT findings and disclosed that epi-on CXL affected the anterior part of the cornea unlike epi-off CXL which induced keratocyte apoptosis and stromal edema in the anterior and middle part of the cornea[31,32] [Figure 6]. At this point, the depth of treatment should be discussed. It is well known that anterior stroma has significantly higher cohesive strength than the middle and posterior corneal stroma[33] and cross-linked of anterior stroma can be enough for strengthening cornea. Recently, Randleman *et al*. compared epi-on CXL and epi-off CXL with optical coherence elastography and observed that epi-on CXL with BAC-EDTA had a greater stiffening effect on the cornea. As a result of present and Randleman *et al*. studies, both techniques are able to stop progression of the disease.[33]

Not only the depth of demarcation line but also the characteristic of demarcation line was different between epi-on and epi-off CXLs. Although the demarcation line was smooth and regular in epi-off CXL, this line was irregular (means that demarcation line was close to the epithelium in some locations and close to the midstroma in some locations [Figure 5] in epi-on CXL. The possible explanation is: if the riboflavin solution is able to pass through the corneal epithelium easily and diffusely, this will induce deeper demarcation line, and if the riboflavin solution is not able to pass or pass limitedly, this will induce superficial demarcation line or insignificant demarcation line.

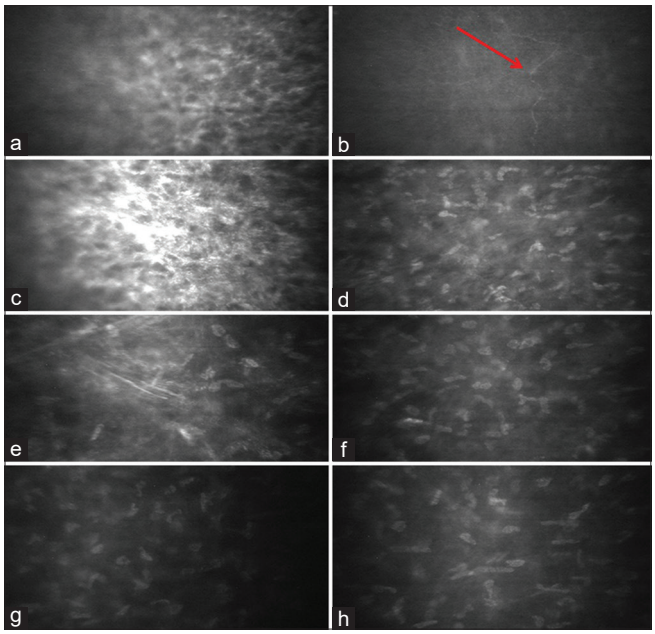

Figure 6: Confocal microscopy; left side is epi-off CXL and right side is epi-off CXL. (a and e) Subepithelial basal membrane, (b and f) anterior stroma, (c and g) midstroma, (d and h) deep stroma, (e) subepithelial nerve (red arrow), (b) intense apoptosis and haze, (f) mild apoptosis, (c) several keratocytes, apoptosis, (g) very mild apoptosis, (d and h) normal deep stroma. CXL=Corneal collagen cross-linking

There were some limitations for this study. The first one is the lack of Schirmer and break-up time tests and the Ocular Surface Disease Index to detect tear film stability. The healing processes are affected with tear function after CXL procedures, and the results may be associated with the impairment of tear function. In addition, the presence of subepithelial nerves in confocal microscopy might affect the tear functions. Second, the keratoconus stage, BCVA, K_{mean}–K_{max}, and CCT were worse in epi-on CXL even the differences were statistically nonsignificant. The nonhomogeneous preoperative levels might affect the results and outcomes of the treatments. However, the contralateral comparison design on the same patients strengthens the study.

Consequently, epi-on CXL was able to stop progression as epi-off CXL; however, in contrast to expectations, the pain was felt more in epi-on CXL than epi-off CXL.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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