

# Iontophoresis-assisted corneal crosslinking using 0.1% riboflavin for progressive keratoconus

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Received: 2016-12-14 Accepted: 2017-02-22

## Abstract

• **AIM:** To report the clinical results of iontophoresis-assisted epithelium-on corneal crosslinking (I-CXL) using 0.1% riboflavin in distilled water for progressive keratoconus.

• **METHODS:** In this prospective clinical study, we examined 94 eyes of 75 patients with progressive keratoconus who were treated with I-CXL using 0.1% riboflavin in distilled water. Best correct visual acuity (BCVA), Scheimpflug tomography, corneal topography, anterior segment optical coherence tomography, intraocular pressure, and endothelial cell density were evaluated at baseline and 1, 3, 6, 12, and 24mo after I-CXL.

• **RESULTS:** After 24mo I-CXL, compared to the level at baseline, BCVA significantly improved  $0.14 \pm 0.07$  ( $P=0.010$ ); mean keratometry significantly decreased  $0.72 \pm 1.97$  ( $P=0.021$ ); maximum keratometry significantly reduced  $2.30 \pm 5.01$  ( $P=0.014$ ); central keratoconus index significantly reduced  $0.04 \pm 0.08$  ( $P=0.007$ ). The demarcation line was visible in 83.1% of eyes at 1mo after treatment, with a depth of  $298.95 \pm 51.97$   $\mu\text{m}$ , and gradually indistinguishable. One eye had repeat treatment. Intraocular pressure and endothelial cell density did not change significantly.

• **CONCLUSION:** I-CXL using 0.1% riboflavin halts keratoconus progression within 24mo, resulting in a significant improvement in visual and topographic parameters. Moreover, the depth of the demarcation line is similar to that previously reported in standard epithelium-off CXL procedures.

• **KEYWORDS:** corneal cross-linking; iontophoresis; keratoconus; distilled water; riboflavin

**DOI:10.18240/ijo.2017.05.09**

Jia HZ, Pang X, Fan ZJ, Li N, Li G, Peng XJ. Iontophoresis-assisted corneal crosslinking using 0.1% riboflavin for progressive keratoconus. *Int J Ophthalmol* 2017;10(5):717-722

## INTRODUCTION

Corneal crosslinking (CXL) is a procedure that improves the biomechanical properties of the corneal stroma; it is currently the only conservative treatment available that can slow or stop the progression of keratoconus<sup>[1]</sup>. The standard technique involves epithelial debridement, which allows riboflavin to penetrate into the corneal stroma, where it leads to the release of reactive oxygen species and the formation of intra- and inter-fibrillary covalent bonds<sup>[2]</sup>. The effectiveness of the standard epithelium-off CXL (S-CXL) procedure in halting keratoconus progression has been demonstrated in many studies with sufficient follow-up<sup>[3-4]</sup> and S-CXL has been recommended as the gold standard of care<sup>[5]</sup>.

However, epithelial removal causes pain, discomfort, and visual loss in the early postoperative period, and carries a risk of corneal infection. Therefore, several transepithelial CXL techniques have emerged. Iontophoresis-assisted transepithelial CXL (I-CXL) is one of these epithelium-on approaches, which increases the imbibition of riboflavin into the corneal stroma with a non-invasive delivery system *via* a small electric current<sup>[6]</sup>. Several prospective case series have demonstrated the stability or improvement of corneal keratometry readings and visual acuity after I-CXL as a treatment for progressive keratoconus. To date, only one prospective randomized controlled trial has been published that compares S-CXL with I-CXL using 0.1% hypoosmotic riboflavin saline buffer and penetration enhancers<sup>[7]</sup>. That study indicated that I-CXL was less effective than S-CXL after 24mo of follow-up, but keratoconus progression was stopped 24mo after treatment.

The main factors affecting CXL efficacy are the intrastromal riboflavin concentration and the total irradiation dose of ultraviolet A (UVA) light. Experimental high performance liquid chromatography (HPLC) studies have shown that intrastromal riboflavin concentrations after 5min of iontophoresis using 0.1% hypoosmotic riboflavin in saline buffer containing penetration enhancers are only half those obtained by passive impregnation after epithelial removal<sup>[8-9]</sup>. However, Li *et al*<sup>[10]</sup> reported that iontophoresis using a 0.1% solution of riboflavin in distilled water resulted in intrastromal riboflavin concentrations similar to those reached after S-CXL.

Here, we report the 24-month follow-up results of our clinical study of I-CXL using 0.1% riboflavin in distilled water as a treatment for progressive keratoconus.

## SUBJECTS AND METHODS

**Patients** In this prospective clinical study, we evaluated 94 eyes of 75 patients with progressive keratoconus. The mean age of the patients was  $20.9 \pm 3.6$  y (range, 16–33y); 69 were male (88 eyes, 93.6%), six were female (six eyes, 6.4%). All patients underwent I-CXL using 0.1% riboflavin in distilled water at the Department of Ophthalmology, PLA Navy General Hospital, Beijing, China, from August 2013 to November 2015. The riboflavin in distilled water was prepared with riboflavin sodium phosphate for injection (Zhuhai Special Economic Zone Biochemical Pharmaceutical Factory, China) and distilled water. All patients provided written informed consent before treatment, and the study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the PLA Navy General Hospital.

Inclusion criteria were age  $\geq 16$ y, a documented progression of disease, the thinnest-point corneal thickness  $\geq 400$   $\mu\text{m}$ , and no obvious corneal scar. Exclusion criteria were a history of intraocular surgery, herpetic keratitis, active keratitis, severe dry eye, or other ocular pathology. Pregnant or breastfeeding patients, and those with concomitant autoimmune diseases, were excluded from the study. Keratoconus progression was defined as a loss of  $\geq 2$  lines of best correct visual acuity (BCVA), or a  $\geq 1.0$ -diopter increase in manifest refraction spherical equivalent, manifest cylinder error, or maximum keratometry (Kmax) attributable just to keratoconus progression in the past 12mo<sup>[11]</sup>.

All patients were evaluated at baseline and at 1, 3, 6, 12, and 24mo after treatment. Examinations comprised pre- and post-treatment BCVA, slit-lamp biomicroscopy, corneal topography (Wavelight, Allegro Topolyzer & Topolyzer Vario, Germany), anterior segment optical coherence tomography (AS-OCT, Spectralis OCT, Heidelberg, Germany), endothelial cell density (SP 2000 noncontact specular microscope, Topcon, Japan) and intraocular pressure. All examinations of the same type were carried out by the same person. All adverse effects during and after treatment were recorded. Rigid gas-permeable contact lens wearers were advised to stop wearing their lenses at least 1wk before treatment and each follow-up visit.

**Surgical Procedures** We carried out iontophoresis-assisted imbibition (SOOFT; IACER Srl, Martellago, Italy) of a 0.1% riboflavin-distilled water solution into the cornea. Topical anesthesia was used. The patient lay supine and the forehead was cleaned with 75% alcohol. The iontophoresis device consisted of a power supply, two electrodes and a connection cable. The negative electrode (an 8-mm-diameter stainless steel grid) was inserted into a rubber ring which was then applied to the cornea using a suction ring, while the positive electrode was connected to the patient's forehead using a patch. After opening the eyelids, an annular suction ring of the iontophoresis device was placed on the cornea. The ring was irrigated with 0.1% riboflavin in distilled water, ensuring

**Table 1 Patient demographics, visual acuity, topographic data, and endothelial biomicroscopy before iontophoresis-assisted corneal crosslinking** ( $n=75$ )

Variables	mean $\pm$ SD
Age (a)	20.9 $\pm$ 3.6
Male/Female	69/6
BCVA (logMAR)	0.45 $\pm$ 0.26
K1 (D)	48.18 $\pm$ 5.05
K2 (D)	53.04 $\pm$ 5.59
Maximum keratometry (D)	62.07 $\pm$ 9.66
Mean keratometry (D)	50.63 $\pm$ 5.11
Thinnest-point corneal thickness ( $\mu\text{m}$ )	456 $\pm$ 37
Astigmatism (D)	4.87 $\pm$ 3.08
Intraocular pressure (mm Hg)	13.52 $\pm$ 3.50
Endothelial cell density (cells/ $\text{mm}^2$ )	2655 $\pm$ 478

BCVA: Best correct visual acuity; D: Diopter; SD: Standard deviation.

the grid was completely immersed. The power generator was then turned on and a constant current of 1.0 mA was selected. Iontophoresis continued for 5min. After saturation of the corneal stroma was confirmed using slit-lamp microscopy, the eye was irradiated for 30min with UVA light (370 nm, 3 mW/ $\text{cm}^2$ ) from a radiation device (UVA Corneal Crosslinking System, Medical Engineering, Colombia, USA). Hypotonic riboflavin drops were applied to the cornea every 2min throughout the procedure.

After treatment, the cornea was immediately rinsed with normothermic saline solution. A bandage contact lens was applied at the end of the procedure. Topical levofloxacin (0.5%), fluorometholone (0.1%), and artificial tears were used for 2wk after I-CXL.

**Statistical Analysis** Decimal visual acuity was converted to the logarithm of the minimal angle of resolution (logMAR). SPSS Statistics 20 (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data are expressed as the mean $\pm$ standard deviation. Paired Student *t*-tests were used to compare pre- and post-treatment data where normal distribution was confirmed, and the Wilcoxon matched-pairs test was used for data with a nonnormal distribution. Two-tailed distribution results were accepted for *P* values. *P*<0.05 was considered statistically significant.

## RESULTS

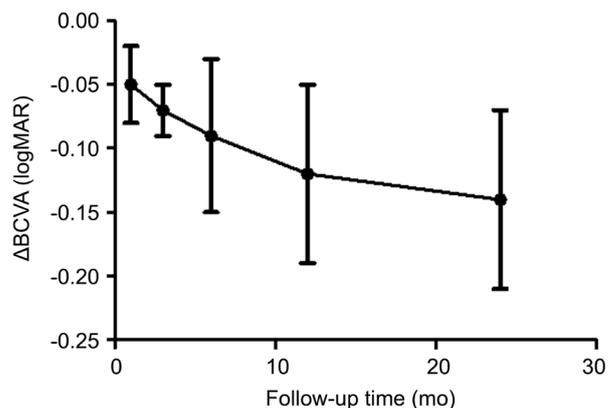
Patient demographics are shown in Table 1. In accordance with the Amsler-Krumeich classification, grade I-IV progressive keratoconus (without stromal scarring) was observed in all 94 eyes: 8 (8.5%) with grade I, 25 (26.6%) with grade II, 45 (47.9%) with grade III, and 16 (17.0%) with grade IV. Only one patient (one eye) needed a second treatment. No serious complications, such as infection or haze, were observed.

**Table 2 Clinical outcomes after iontophoresis-assisted corneal crosslinking compared with baseline measurements** mean±SD

Parameters	1mo	3mo	6mo	12mo	24mo	P
No. of eyes (%)	78 (83.0)	63 (67.0)	68 (72.3)	46 (48.9)	38 (40.4)	-
ΔBCVA (logMAR)	-0.05±0.03	-0.07±0.02	-0.09±0.06 <sup>a</sup>	-0.12±0.07 <sup>a</sup>	-0.14±0.07 <sup>a</sup>	0.010 <sup>a</sup>
ΔK1 (D)	-0.17±1.64	-0.67±2.27 <sup>a</sup>	-0.84±2.56 <sup>a</sup>	-0.72±2.31 <sup>a</sup>	-0.82±2.04 <sup>a</sup>	0.015 <sup>a</sup>
ΔK2 (D)	-0.13±1.82	-0.67±1.90 <sup>a</sup>	-1.11±2.42 <sup>a</sup>	-0.76±2.11 <sup>a</sup>	-1.36±2.02 <sup>a</sup>	0.025 <sup>a</sup>
ΔKmax (D)	-0.66±2.81 <sup>a</sup>	-1.40±4.04 <sup>a</sup>	-2.33±5.35 <sup>a</sup>	-2.46±4.63 <sup>a</sup>	-2.30±5.01 <sup>a</sup>	0.014 <sup>a</sup>
ΔKmean (D)	-0.22±1.39	-0.68±2.01 <sup>a</sup>	-0.95±2.38 <sup>a</sup>	-0.77±2.19 <sup>a</sup>	-0.72±1.97 <sup>a</sup>	0.021 <sup>a</sup>
ΔISV	0.62±11.53	-4.13±17.17	-6.47±24.17 <sup>a</sup>	-7.19±23.16 <sup>a</sup>	-12.28±27.75 <sup>a</sup>	0.018 <sup>a</sup>
ΔIHA	-4.79±27.88	-4.00±29.65	-1.71±24.75	-5.82±31.05	-6.42±29.12	0.222
ΔIVA	0.16±1.16	-0.01±0.24	-0.15±1.16	-0.01±0.28	-0.01±0.33	0.861
ΔIHD	-0.01±0.07	-0.01±0.03	-0.02±0.09	-0.02±0.09	-0.01±0.04	0.131
ΔKI	0.01±0.07	0.01±0.11	0.00±0.09	0.02±0.09	-0.01±0.09	0.419
ΔCKI	-0.01±0.03	-0.01±0.04	-0.02±0.08	-0.02±0.07	-0.04±0.08 <sup>a</sup>	0.007 <sup>a</sup>
ΔRmin (mm)	0.07±0.25 <sup>a</sup>	0.14±0.31 <sup>a</sup>	0.17±0.43 <sup>a</sup>	0.21±0.46 <sup>a</sup>	0.36±0.56 <sup>a</sup>	0.001 <sup>a</sup>
ΔTCT (μm)	-30±36 <sup>a</sup>	-28±35 <sup>a</sup>	-24±34 <sup>a</sup>	-17±24 <sup>a</sup>	-16±18 <sup>a</sup>	0.006 <sup>a</sup>
ΔAstigmatism (D)	0.02±1.97	-0.01±1.40	-0.29±1.84	-0.04±1.93	-0.14±1.53	0.116
ΔIOP (mm Hg)	-0.17±4.30	-0.56±2.96	-0.13±3.22	0.42±3.90	1.25±3.05	0.183
ΔECD (cells/mm <sup>2</sup> )	-63±434	-60±425	-71±503	-56±488	163±580	0.372
Demarcation line (%)	83.3	31.7	5.9	0	0	

Δ: Change from baseline; BCVA: Best correct visual acuity; logMAR: Logarithm of the minimum angle of resolution; K1: Corneal dioptric power in the flattest meridian for the 3-mm central zone; K2: Corneal dioptric power in the steepest meridian for the 3-mm central zone; Kmax: Maximum keratometry; Kmean: Mean keratometry; D: Diopter; ISV: Index of surface variance; IVA: Index of vertical asymmetry; KI: Keratoconus index; CKI: Central keratoconus index; IHA: Index of height asymmetry; IHD: Index of height decentration; Rmin: Minimum radius of curvature; TCT: Thinnest-point corneal thickness; IOP: Intraocular pressure; ECD: Endothelial cell density; <sup>a</sup>P<0.05 vs baseline.

Table 2 shows the results at each follow-up visit. There was a significant improvement from baseline in BCVA at 6, 12, and 24mo ( $P=0.032$ ,  $0.021$ , and  $0.010$ , respectively); at 1 and 3mo follow-up, BCVA improvement was not significant (Figure 1). Similarly, flat (K1) and steep (K2) corneal dioptric power and mean keratometry were significantly lower than baseline at 3, 6, 12, and 24mo (Figure 2). Kmax was also significantly reduced at 1mo and remained lower than baseline for the duration of the study period (Figure 3). The index of surface variance was lower than baseline at 6mo and continued to decrease over time. Central keratoconus index decreased  $0.04±0.08$  significantly at the last follow-up ( $P=0.007$ ). Minimum radius of curvature significantly increased  $0.36±0.56$  than baseline at 24mo ( $P=0.001$ ) (Figure 4). Indices of height asymmetry, vertical asymmetry, and height decentration remained stable for the entire study period, as did the keratoconus index and corneal astigmatism values. The thinnest-point corneal thickness (TCT) was significantly lower than baseline at 1mo, then increased for the remainder of the study period but did not return to the pre-treatment level ( $P=0.006$ ) (Figure 5). Intraocular pressure and endothelial cell density did not change significantly during the follow-up period.



**Figure 1 Change from baseline in best correct visual acuity (ΔBCVA) after I-CXL.**

The depth of the stromal demarcation line 1mo after I-CXL (Figure 6) was  $298.95±51.97$  μm in 65 of 78 eyes and could not be seen in the remaining 16.7%. It became indistinguishable over time (Figure 7); at 3mo it was measureable in 20 of 63 eyes, and at 6mo it was measureable, but very faint, in 4 of 68 eyes.

**DISCUSSION**

The present study shows that I-CXL using 0.1% riboflavin in distilled water halts the progression of keratoconus within 24mo, and results in statistically significant improvements in

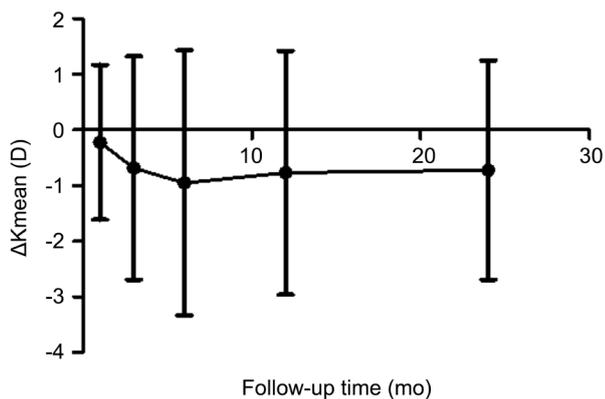


Figure 2 Change from baseline in mean keratometry ( $\Delta K_{\text{mean}}$ ) after I-CXL.

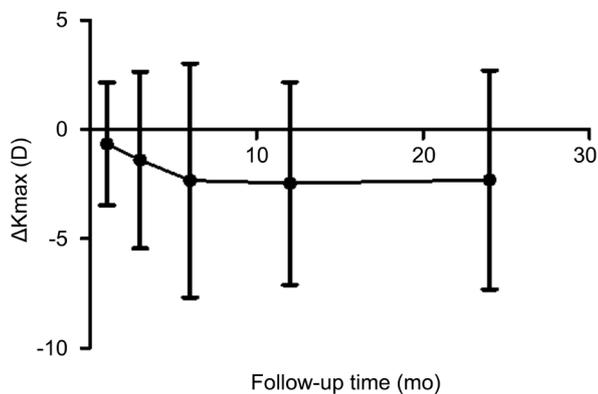


Figure 3 Change from baseline in maximum keratometry ( $\Delta K_{\text{max}}$ ) after I-CXL.

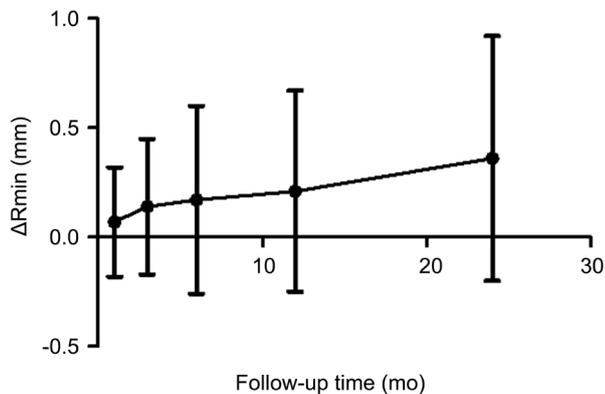


Figure 4 Change from baseline in minimal curvature radius ( $\Delta R_{\text{min}}$ ) after I-CXL.

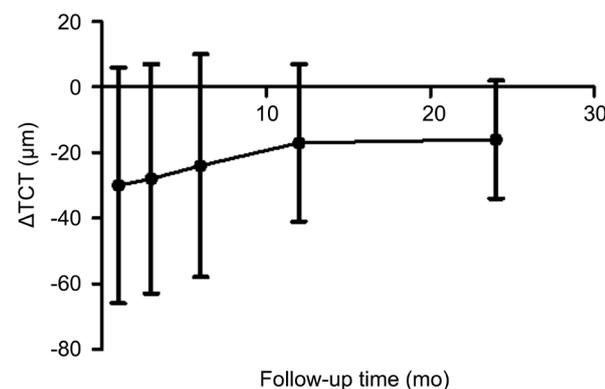


Figure 5 Change from baseline in thinnest-point corneal thickness ( $\Delta TCT$ ) after I-CXL.

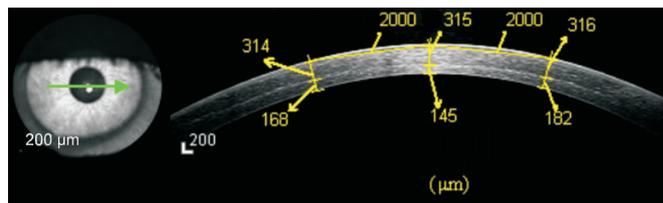


Figure 6 Demarcation line (depth, 315  $\mu\text{m}$ ) on anterior segment OCT image 1mo after iontophoresis-assisted corneal crosslinking.

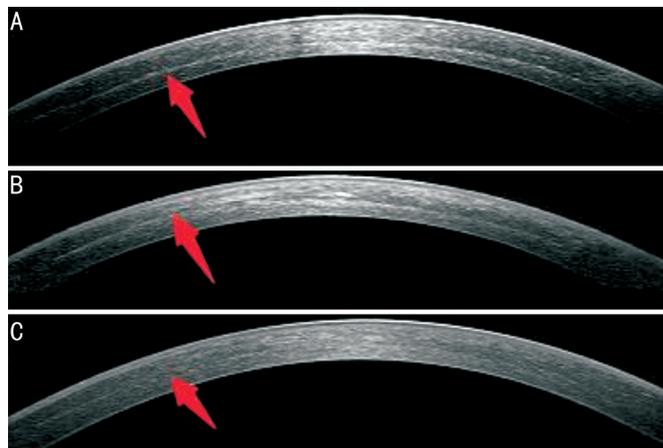


Figure 7 Anterior segment OCT image at 1 (A), 3 (B), and 6mo (C) after I-CXL. The demarcation line became indistinguishable over time.

visual and topographic parameters. Furthermore, endothelial cell density remains stable throughout the study, indicating that the procedure is safe.

Corneal intrastromal riboflavin concentration is one of the most important factors affecting crosslinking efficacy. To date, most clinical studies of I-CXL have used Ricrolin+, a hypoosmolar 0.1% riboflavin solution enriched with penetration enhancers, without dextran or sodium chloride, and specifically formulated to facilitate quick penetration into the corneal stroma *via* an intact epithelium. Iontophoresis-assisted transepithelial imbibition with Ricrolin+ yields greater and deeper riboflavin saturation than the conventional epithelium-on protocol, but does not reach the level acquired with the standard epithelium-off protocol<sup>[8-9,12-13]</sup>. Here, we used a solution of 0.1% riboflavin in distilled water during iontophoresis delivery. Li *et al*<sup>[10]</sup> investigated the imbibition of 0.1% riboflavin in distilled water into the corneal stroma of rabbits using iontophoresis-assisted delivery with a 1 mA current for 10min, and obtained similar intrastromal riboflavin concentrations to those observed with the standard protocol. This might be due to the presence of fewer parasitic ions in the distilled water, resulting in less interference with riboflavin permeability; or to the hypoosmotic pressure in the riboflavin-distilled water solution, which damages the barrier function of the epithelium. In the present study, five minutes was enough to obtain the same results in patients.

Li *et al*<sup>[14]</sup> reported that there were no statistically significant changes in BCVA, corneal curvature, or TCT 6mo after the same I-CXL protocol. In contrast, at the same time point, we

observed a significant improvement in BCVA, and significant decreases in corneal curvature and TCT. These conflicting results were probably due to methodological differences. Fewer participants were enrolled in the study by Li *et al*<sup>[14]</sup>, and the authors did not specify which stages of keratoconus were included. In addition, the efficacy of CXL is affected by age, and pre-treatment BCVA and TCT<sup>[15]</sup>.

There have been several other clinical studies investigating the efficacy of I-CXL using a hypoosmolar 0.1% riboflavin solution enriched with penetration enhancers without dextran or sodium chloride. Some included only patients with keratoconus stages I-II<sup>[6-7,16]</sup>, others included stages I-IV, similar to our patient group<sup>[17-18]</sup>, and others did not mention stages<sup>[1,19-20]</sup>. Two studies investigated only pediatric keratoconus<sup>[16,18]</sup>. In all these studies, the postoperative BCVA and topographic indices (keratometry values) improved or remained stable. However, only one report, by Bikbova and Bikbov<sup>[7]</sup>, showed a significant improvement of both BCVA and topographic indices, which is in line with our results, despite the difference in stages examined.

After S-CXL, a corneal stromal demarcation line is visible on AS-OCT images as early as 2wk at a depth of 281-313  $\mu\text{m}$ <sup>[7,21-25]</sup>. This line has been attributed to ultrastructural changes in stromal collagen<sup>[22]</sup>. The depth of the demarcation line is considered to be related to the effectiveness of the CXL procedure<sup>[22-23]</sup> because deeper crosslinking induces greater collagen changes and improves greater corneal biomechanical properties. A deeper demarcation line indicates a deeper treatment and more cross-linked stromal tissue; the depth of the line is significantly related to the change in Kmean<sup>[25]</sup>. The corneal stromal demarcation line was at a depth of 298.95 $\pm$ 51.97  $\mu\text{m}$  in our study and 288.46 $\pm$ 30.00  $\mu\text{m}$  in the report by Li *et al*<sup>[14]</sup> in which the same riboflavin solution was used; these are comparable to the results obtained with S-CXL. In other studies on I-CXL<sup>[1,6-7,16,19-20]</sup>, the depth of the demarcation line was 169-246.67  $\mu\text{m}$  lower than that in S-CXL, and Vinciguerra *et al*<sup>[17]</sup> did not observe any clear demarcation line with AS-OCT after I-CXL. Bikbova and Bikbov<sup>[7]</sup> reported that a demarcation line was measureable in only 47% of patients 1mo after I-CXL and completely disappeared in all these patients after 3mo, whereas in the S-CXL group it was measureable in 96% of patients after 1mo, 66% after 3mo, and 48% after 6mo. In the present study, at the same time points, this line was measureable in 83.3%, 31.7%, and 5.9% of eyes, respectively. In our study, the line was present in fewer patients, and faded more quickly, than after S-CXL, but was present in more patients, and faded more slowly, than that reported by Bikbova and Bikbov<sup>[7]</sup> after I-CXL. The proportion of patients with a demarcation line in S-CXL and I-CXL 1mo postoperatively in another two studies<sup>[19-20]</sup> was similar to that observed by Bikbova and Bikbov<sup>[7]</sup>.

In our study, TCT was lower than baseline 1mo after treatment and then increased, although it did not reach the pre-treatment level by the end of the study period. Changes in corneal thickness after CXL have also been reported in other studies using S-CXL<sup>[7,26-27]</sup> and I-CXL<sup>[6-7]</sup>; some showed that TCT or corneal central thickness remained stable after I-CXL<sup>[16-19]</sup>, and a decrease in TCT was also used as an indicator of preoperative keratoconus progression<sup>[17,19]</sup>. However, a decrease in TCT after treatment did not necessarily indicate progression of the condition, which was usually accompanied by an increase in corneal curvature and impaired vision. Furthermore, TCT increased slowly after 1mo. The reason for a decrease in corneal thickness after CXL was probably stromal collagen fiber compression and rearrangement induced by collagen crosslinking<sup>[28-29]</sup>. The degree of corneal thinning after CXL can be considered to represent the intensity of crosslinking. In their randomized controlled trial comparing S-CXL and I-CXL, Bikbova and Bikbov<sup>[7]</sup> found that after S-CXL, TCT was less than baseline levels significantly at 24mo; whereas in their I-CXL group, TCT showed no significant changes at 24mo compared to the baseline level. In other words, by 24mo after treatment, collagen fiber compression and rearrangement in the S-CXL group was greater than that in the I-CXL group; indeed, keratometry values showed that S-CXL was more effective than I-CXL. In our study, TCT did not recover to the pre-treatment level by 24mo, which may suggest that the intensity of crosslinking was greater than that in the I-CXL group in the randomized controlled trial<sup>[7]</sup>.

The depth of effective CXL depends on the intensity of UVA light and corneal intrastromal riboflavin concentration; oxygen is also an essential element for photopolymerization in CXL to occur<sup>[30]</sup>. An intact corneal epithelium may result in less photopolymerization after I-CXL because there is less rapid oxygen diffusion, and it acts as a UVA barrier, filtering out 20% of the radiation<sup>[31]</sup>. In addition, riboflavin supply reduces during UVA irradiation as a result of the removal of the iontophoresis device. Although corneal intrastromal riboflavin concentration after transepithelial iontophoresis delivery can, in theory, reach the level obtained by the standard epithelium-off protocol, the efficacy of I-CXL may also be inferior to that of S-CXL in practice. However, it is not yet clear exactly what conditions are necessary to achieve sufficient crosslinking.

A limitation of our study is the high lost rate of follow-up. Only 38 in 94 eyes were followed up for 24mo. Another limitation of our study is the follow-up time limited to 24mo, also lacking the comparison with S-CXL.

The results of the present study are promising, but a longer follow-up period is required to assess the long-term outcomes of I-CXL using 0.1% riboflavin in distilled water. In addition, a randomized controlled trial comparing S-CXL and I-CXL using the same solution is necessary. Further research is needed into how to supply riboflavin to the corneal stroma during UVA irradiation and whether to increase the intensity of UVA irradiation by 20% in I-CXL.

**ACKNOWLEDGEMENTS**

**Foundation:** Supported by Beijing Municipal Science and Technology Commission (No. Z151100004015217).

**Conflicts of Interest:** Jia HZ, None; Pang X, None; Fan ZJ, None; Li N, None; Li G, None; Peng XJ, None.

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