Int Ophthalmol (2019) 39:1089–1095
https://doi.org/10.1007/s10792-018-0920-4

ORIGINAL PAPER

Trans-epithelial corneal collagen cross-linking with iontophoresis for progressive keratoconus

Mohammed Ali Abu Ameerh · Muawyah Dawoud Al Bdour · Maha Al-Till · Muhannad Al Faouri

Received: 19 September 2017 / Accepted: 7 April 2018 / Published online: 19 April 2018

Abstract

Purpose To evaluate the efficacy of trans-epithelial corneal collagen cross-linking (CXL) with Iontophoresis among patients with progressive keratoconus.

Methods It is a prospective interventional study, which is based on 41 eyes of 23 patients, suffering from progressive keratoconus and treated with trans-epithelial corneal cross-linking, using iontophoresis with ETDA and trometamol-enriched riboflavin 5 phosphates 0.1% hypotonic solution (Ricrolin+, Soot Italia SpA, Italy).

Results The mean of uncorrected distance visual acuity and best corrected distant visual acuity was improved at 6 months with statistically significant differences from baseline ($p < 0.05$). There was no statistically significant difference in keratometric values, including K_1, K_2, K_m, topographic astigmatism, and central corneal thickness. Patients, who had completed 1 year (21 eyes of 12 patients) of the treatment, showed similar results.

Conclusion The data indicated that corneal collagens cross-linking with iontophoresis using Ricroli+ may be an effective method in halting the progression of keratoconus without the side effects of epithelial removal, which may be encountered in the standard epi-off CXL procedure.

Keywords Corneal cross-linking · Corneal thickness · Iontophoresis · Keratoconus · Trans-epithelial cross-linking

M. A. A. Ameerh (✉) · M. D. A. Bdour
Ophthalmology Department, The University of Jordan, P.O Box 13046, Amman 11942, Jordan
e-mail: mohammd_73@yahoo.com

M. Al-Till · M. A. Faouri
Al Taif Eye Center, Amman, Jordan

Introduction

Keratoconus is a non-inflammatory process that results in paracentral corneal thinning and secondary ectasia, which causes irregular astigmatism with impaired vision [1]. Keratoconus progresses between the second and fifth decades of life and may ultimately require corneal transplantation in 10 to 20% of the cases [2]. The treatment of corneal collagen cross-linking using Riboflavin (vitamin B2) and Ultraviolet-A (UV-A) (370 nm) Radiation induces covalent bonds between collagen fibers. The naturally occurring protein cross-linking accelerates with age. However, strengthening and stiffening of the cornea was first introduced at the University of Dresden by Spoerl and Seiler in porcine corneas in 1998 [3].

Wollensak et al. [4] were the first one to describe a clinical method of corneal cross-linking in non-randomized pilot study, conducted among 22 patients. The study reported a halt in the progression of all treated eyes [4]. Thereafter, several studies have

demonstrated the efficacy of corneal cross-linking in halting the progression of keratoconus [5–11]. The largest trial including 241 eyes was conducted by Raiskup-Wolf et al. [6]. The treatment of corneal collagen cross-linking may decrease the steepness of cone and improve uncorrected (UDVA), corrected (CDVA) distance visual acuities, and subjective visual symptoms [12–15].

Vinciguerra et al. [16] demonstrated that key feature of keratoconus is a loss of corneal mechanical stability that leads to a decrease in stiffness of the cornea by 60% than the healthy state. Similarly, the study reveals an improvement to the soaking time with respect to the diffusion of riboflavin and treatment time reduction due to the iontophoresis capacity. Zhang et al. [17] showed a gradual recovery in the epithelial thickness during the first operative period. As proposed, the accelerated trans-epithelial technique mitigates the impact of risk complications resulting from epithelial removal and assures the stability of keratoconus too. Arleta [18] treats the corneal cross-structure through standard cross-linking methodology by involving corneal epithelium removal for enhancing riboflavin penetration.

Majority of the trials, treating corneal collagen cross-linking, used the standard technique Epi-Off Dresden protocol, which involves the removal of central corneal epithelium to allow maximum penetration of riboflavin into the corneal stroma. In Epi-On trans-epithelial corneal collagen cross-linking, the corneal epithelium is not removed; so that, it may increase the safety profile by reducing the risk of infection and improve postoperative patient comfort.

Aim of the study

The study aims to evaluate the safety and efficacy of epi-on trans-epithelial corneal treatment using iontophoresis technique. This study was conducted at Al-Taif eye center/Amman, Jordan.

Materials and methods

The prospective clinical study was conducted at Al-Taif eye center/Amman, Jordan, from February 2015 to January 2017. The study evaluated 41 eyes of 23 patients with progressive keratoconus. The mean age of the patients was 31.05 ± 6.64 years. Out of the 23 patients, 7 were male (30.4%) and 16 (69.6%) were female. All the patients were followed up after 6 and 12 months of the treatment. Examinations were based on pre- and post-BCVA, K_1, K_2 and K_m under the topographic outcomes. After the treatment, the patients were followed up for 1 year. At each follow-up visit, a standard examination was carried out to assess uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), refractometry, keratometry, and corneal topography using pentacam. The progression of keratoconus over 1 year was defined on the basis of following changes;

- Increase in steepest K by 1.0 diopter (D) or more in manifest cylinder.
- Increase of 0.5 D or more in manifestation of spherical equivalent refraction by repeated keratotopography using pentacam.

Surgical technique

All the eyes were treated with trans-epithelial corneal cross-linking using iontophoresis under topical anesthesia.

Surgical procedure

The anesthetic eye drops (oxybuprocaine hydrochloride 0.4%, Novesina, Novartis Farma) were instilled 3 times over a 10-min period before each treatment. A lid speculum was inserted, and Biopore membrane attached to a plastic cylinder was pressed against the central cornea. The membrane was pressed with sufficient pressure to applanate the central cornea for 3 s, and then the precorneal mucin layer was removed. Corneal soaking with ETDA and trometamol-enriched riboflavin 5 phosphate 0.1% hypotonic solution (Ricrolin+, Soot Italia SpA, Italy) was performed using a commercial iontophoresis device (Iontophor CXL, Soot Italia SpA, Italy). The passive electrode was applied to forefront of the eye to be treated. The active electrode was applied to the corneal surface. The tube was filled with riboflavin solution after suctioning it to the corneal epithelium.

The current intensity of the treatment was set at 1.0 mA for 5 min. The corneal surface was gently washed with chilled 0.9% sodium chloride solution after iontophoresis. Corneal UV-A irradiation was then applied using 10 mW/cm^2 device at 56 mm

distance for 9 min. One drop of chilled 0.9% sodium chloride solution was applied over the corneal epithelium every 3 min during irradiation. After the procedure, no steroidal eye drops were installed and a bandage contact lens was applied after giving topical antibiotic. Same procedure is performed on the other eye in case of bilateral treatment.

Statistical analysis

Statistical analysis was performed using SPSS (Version 17). The characteristics of participants were investigated by calculating frequencies, percentages, means, and standard deviations. Paired sample t test was carried out to explore differences between preoperative and postoperative examinations at significance level of ($p < 0.05$). The effect size was calculated to discover whether the effect is substantive or not.

Results

Forty-one eyes of 23 patients underwent corneal collagen cross-linking using trans-epithelial iontophoresis with Ricrolin+ (Ricrolin+, Soot Italia SpA, Italy). All the 23 patients completed the 6-month follow-up with a total of 41 treated eyes. Among 23 patients, 12 of the initially enrolled patients completed the 1-year follow=-up, including 5 males (42.7%) and 7 females (58.3%) with a total of 21 treated eyes (Table 1).

At 6 months, the results showed a statistically significant improvement in both uncorrected distance visual acuity (UDVA) and best corrected distance visual acuity (BCDVA). Mean postoperative values were 0.31 and 0.67 Snellen equivalent at 6 months, which were compared with 0.21 and 0.57 Snellen equivalent at baseline with p values of 0.000 and 0.42, respectively. Comparison of topographic data revealed no statistically significant differences in K_1, K_2, and K_m between baseline and 6 months. Baseline K_1, K_2, and K_m were 46.48, 51.05, and 49.36 diopter, respectively, that were compared with 47.72, 51.38, and 49.43 diopter at 6 months. The p values for K_1, K_2, and K_m were calculated to be 0.737, 0.369, and 0.499, respectively. The results illustrated the stable topographic astigmatism and the thinnest corneal thickness at 6 months by comparing it with baseline. The mean value of topographic astigmatism at baseline was 3.52 diopter, which was compared with 3.49 diopter at 6 months with p value = 1.00. The baseline value for thinnest corneal thickness was 452.22 microns that was compared with 451.7 31 microns at 6 months (p values = 0.872). Table 2 summarizes the visual and topographic outcomes of 6 months that were obtained by trans-epithelial collagen cross-linking by iontophoresis with Ricrolin+.

Table 1 Patients' demographics

	Frequency	Percentage
Gender		
Male	7	30.4
Female	16	69.6
Age		
Mean age of all patients	31.05 ± 6.64 years	–
One-year follow-up		
Male	5	42.7
Female	7	58.3

At 12 months, only 12 patients (21 eyes) completed the 1-year follow-up. The results indicated that patients had significantly higher uncorrected visual acuity (0.367) compared with (0.202) Snellen equivalent preoperatively (p value = 0.000) after 1 year. The results also showed a slight improvement in the best corrected distance visual acuity (0.728) compared with (0.595) Snellen equivalent preoperatively (p value = 0.116). For topographic values (K_1, K_2, and K_m), no statistically significant differences were found between baseline and 1 year. K_1, K_2, and K_m values after 1 year were 46.847, 50.476, and 48.571 diopter, respectively. These values were compared with 46.480, 49.961, and 48.133 diopter, respectively at baseline with p values of 0.077, 0.126, and 0.802 respectively. No statistically significant differences were found in the topographic astigmatism nor thinnest corneal thickness with postoperative mean values of 3.619 diopter and 461 microns, p values of 0.504 and 0.00, respectively. Table 3 has summarized 1 year visual and topographic outcomes that were obtained by trans-epithelial collagen cross-linking by iontophoresis with Ricrolin+. The mean difference was 0.051 among the patients underwent Snellen test for UCVA. In contrast, a mean difference of 0.058 was measured for the BCDVA among the patients

Table 2 Six-month visual and topographic outcomes obtained by trans-epithelial collagen cross-linking by iontophoresis with Ricrolin+

	Snellen equivalent at baseline	Snellen equivalent at 6 months	*p* value
Visual outcomes			
UDVA	0.21	0.31	0.000
BCDVA	0.57	0.67	0.42
	Baseline	At 6 months	*p* value
Topographic outcomes			
K_1	46.48	47.72	0.737
K_2	51.05	51.38	0.369
K_m	49.36	49.43	0.499

Table 3 One-year visual and topographic outcomes obtained by trans-epithelial collagen cross-linking by iontophoresis with Ricrolin+

	Baseline	At 1 year	*p* value	Mean difference for Snellen test after 6 and 12 months
Visual outcomes				
Uncorrected visual acuity	0.202	0.367	0.000	0.051
Best corrected distance visual acuity	0.595	0.728	0.116	0.058
	Baseline	At 1 year	*p* value	Mean difference between topographic outcomes after 6 and 12 months
Topographic outcomes				
K_1	46.480	46.847	0.077	− 0.873
K_2	49.961	50.476	0.126	− 0.904
K_m	48.133	48.571	0.802	− 0.859

underwent Snellen test after 12 months as compared to 6 months.

Postoperatively, none of the patients complained about early postoperative pain or increased glare as it can be seen with the standard epi-off cross-linking technique. None of the patients developed significant corneal haze or keratitis during the follow-up at 6 months and 1 year.

Discussion

Corneal collagen cross-linking halts the progression of keratoconus by creating new covalent bonds between collagen fibers; so that, it strengthens cornea and increases its rigidity by 300% [19]. In some cases, CXL improves the refractive and topographic features of treated corneas [20, 21]. Most of complications related to standard CXL are associated with epithelial removal. It may even include significant postoperative pain, infection, stromal haze, and corneal melting [22–24]. A trans-epithelial CXL with different technique has been suggested to improve safety, reduce treatment time, and avoid serious side effects to maintain same efficacy of standard epi-off procedure. Shalchi et al. [25] compared the results of standard epi-off CXL with a total of 45 papers and trans-epithelial CXL with a total of 5 papers in the management of progressive keratoconus. Majority of studies on standard CXL have shown reduction in maximum simulated keratometry; whereas, trans-epithelial corneal CXL did not halt keratoconus progression in about 75% of cases within 1 year [26].

In the present study, mean preoperative UCDVA was increased from 0.20 to 0.31 (p value = 0.000), and BCDVA increased from 0.59 to 0.670 (p value = 0.042.). The patients, who completed 1 year, showed more improvement for UCDVA and

BCDVA, which was statistically significant. Mean UCDVA was increased from 0.20 to 0.38 (p value = 0.000); and BCDVA increased from 0.59 to 0.73 (p value = 0.116). Bottos et al. [26] showed that the reduced effect of trans-epithelial CXL as compared to standard CXL is principally due to the limited penetration of riboflavin through the epithelium. The study also confirmed that corneal epithelium is not a barrier to ultraviolet-A transmittance [27]. A few studies have reported the efficacy of trans-epithelial CXL with iontophoresis in halting the progression of keratoconus [28, 29]. These studies have reported an improvement in BCDVA, stable keratometric values, no changes in central corneal thickness, and no modifications in endothelial cell count during the 1-year follow-up [30, 31].

Two randomized clinical trials have compared trans-epithelial CXL with standard epi-off CXL procedure using Iontophoresis. The results concluded that trans-epithelial CXL with Iontophoresis might be comparable to standard epi-off procedure to stabilize the progression of keratoconus at 6 months [31] and 1 year [32]. The findings of present study are in agreement with previous reports on trans-epithelial CXL with Iontophoresis [28–30]. The findings have shown functional improvements observed in both UCDVA and BCDVA. These findings were inline with the findings of Arleta [18] who examined keratometric stability at 6 and 12 months in terms of both UCDVA and BCDVA. Similarly, Jia et al. [32] have shown a significant improvement in the keratoconus at 6, 12 and 24 months as compared to baseline in BCDVA. Furthermore, there was significant difference for the flat (K_1) and steep (K_2) corneal dioptric power after 6, 12 and 24 months as compared to baseline. However, there was insignificant mean difference for the flat (K_1) and steep (K_2) corneal dioptric power after 6 and 12 months as compared to the baseline. Bikbova and Bikbov [33] have shown significant mean differences between 6 and 24 months in CDVA as compared to the baseline, which is inline with the present study findings at 6 and 12 months. The results have also shown keratometric stability at 6 months and 1 year, whereas functional improvements have been observed in both UCDVA and BCDVA.

Conclusion

The results supported the concept that CXL with Iontophoresis is effective in halting the progression of keratoconus without the side effects of epithelial removal, which may be encountered in the standard epi-off CXL procedure. The study has evaluated trans-epithelial Iontophoresis treatment with Ricroli+ (Ricrolin+, Soot Italia SpA, Italy) using Biopore membrane with removal of mucin layer for progressive keratoconus in 23 patients with a total of 41 eyes. A total energy dose of 5.4 J/cm^2 was applied. All treated eyes 41 eyes were followed up for 6 months, and only 12 patients with 21 eyes completed 1 year. At 6 months, both uncorrected (UCDVA) and best corrected distance visual acuity (BCDVA) were significantly improved. Mean topographic values including K_1, K_2, K_m, and topographic astigmatism were stable at 6 months and 1 year for those completed the 1-year follow-up. These results indicated that CXL with Iontophoresis is effective in halting the progression of keratoconus among patients. However, the results are limited due to lack of control group, a relatively small number of patients, and the short follow-up period. These factors are not sufficient to judge the safety and efficacy of this trans-epithelial approach with Iontophoresis.

Acknowledgements The authors are very thankful to all the associated personnel in any reference that contributed in/for the purpose of this research. This study was conducted at Al-Taif eye center, Amman, Jordan.

Author contribution The authors have analyzed the effectiveness of trans-epithelial corneal collagen cross-linking with Iontophoresis for treating progressive keratoconus.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All the patients signed an informed written consent, and the study adhered to the tenets of the Declaration of Helsinki.

References

1. Krachmer JH, Feder RS, Belin MW (1984) Keratoconus and related noninflammatory corneal thinning disorders. Surv Ophthalmol 28:293–322. https://doi.org/10.1016/0039-6257(84)90094-8

2. Tuft SJ, Moodaley LC, Gregory WM, Davison CR, Buckley RJ (1994) Prognostic factors for the progression of keratoconus. Ophthalmology 101:439–447. https://doi.org/10.1016/s0161-6420(94)31313-3
3. Spoerl E, Huhle M, Seiler T (1998) Induction of cross-links in corneal tissue. Exp Eye Res 66:97–103. https://doi.org/10.1006/exer.1997.0410
4. Wollensak G, Spoerl E, Seiler T (2003) Riboflavin/ultraviolet-A–induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 135:620–627. https://doi.org/10.1016/s0002-9394(02)02220-1
5. Kampik D, Koch M, Kampik K, Geerling G (2011) Corneal riboflavin/UV-A collagen cross-linking (CXL) in keratoconus: two-year results. Klin Monatsblatter Augenheilkd 228:525–530. https://doi.org/10.1055/s-0031-1273403
6. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE (2008) Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. J Cataract Refract Surg 34:796–801. https://doi.org/10.1016/j.jcrs.2007.12.039
7. Saffarian L, Khakshoor H, Zarei-Ghanavati M, Esmaily H (2010) Corneal crosslinking for keratoconus in Iranian patients: outcomes at 1 year following treatment. Middle East Afr J Ophthalmol 17:365. https://doi.org/10.4103/0974-9233.71600
8. Koller T, Pajic B, Vinciguerra P, Seiler T (2011) Flattening of the cornea after collagen crosslinking for keratoconus. J Cataract Refract Surg 37:1488–1492. https://doi.org/10.1016/j.jcrs.2011.03.041
9. Asri D, Touboul D, Fournie P, Malet F, Garra C, Gallois A, Malecaze F, Colin J (2011) Corneal collagen crosslinking in progressive keratoconus: multicenter results from the French National Reference Center for Keratoconus. J Cataract Refract Surg 37:2137–2143. https://doi.org/10.1016/j.jcrs.2011.08.026
10. Derakhshan A, Shandiz JH, Ahadi M, Daneshvar R, Esmaily H (2011) Short-term outcomes of collagen crosslinking for early keratoconus. J Ophthal Vis Res 6:155
11. Viswanathan D, Males J (2013) Prospective longitudinal study of corneal collagen cross-linking in progressive keratoconus. Clin Exp Ophthalmol 41:531–536. https://doi.org/10.1111/ceo.12035
12. Hersh PS, Greenstein SA, Fry KL (2011) Corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. J Cataract Refract Surg 37:149–160. https://doi.org/10.1016/j.jcrs.2010.07.030
13. Brooks NO, Greenstein S, Fry K, Hersh PS (2012) Patient subjective visual function after corneal collagen crosslinking for keratoconus and corneal ectasia. J Cataract Refract Surg 38:615–619. https://doi.org/10.1016/j.jcrs.2011.11.029
14. Greenstein SA, Fry KL, Hersh MJ, Hersh PS (2012) Higher-order aberrations after corneal collagen crosslinking for keratoconus and corneal ectasia. J Cataract Refract Surg 38:292–302. https://doi.org/10.1016/j.jcrs.2011.08.041
15. Greenstein SA, Fry KL, Hersh PS (2011) Corneal topography indices after corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. J Cataract Refract Surg 37:1282–1290. https://doi.org/10.1016/j.jcrs.2011.01.029
16. Vinciguerra P, Romano V, Rosetta P, Legrottaglie EF, Kubrak-Kisza M, Azzolini C, Vinciguerra R (2016) Iontophoresis-assisted corneal collagen cross-linking with epithelial debridement: preliminary results. Biomed Res Int. https://doi.org/10.1155/2016/3720517
17. Zhang X, Sun L, Chen Y, Li M, Tian M, Zhou X (2016) One-year outcomes of pachymetry and epithelium thicknesses after accelerated (45 mW/cm^2) Transepithelial corneal collagen cross-linking for keratoconus patients. Sci Rep. https://doi.org/10.1038/srep32692
18. Arleta W (2018) Corneal cross-linking procedure in progressive keratoconus treatment. Biomed J Sci Tech Res 2(3). BJSTR.MS.ID.000754. https://doi.org/10.26717/bjstr.2018.02.000754
19. Sporl E, Huhle M, Kasper M, Seiler T (1997) Artificial stiffening of the cornea by induction of intrastromal crosslinks. Der Ophthalmol 94:902–906. https://doi.org/10.1007/s003470050219
20. Caporossi A, Baiocchi S, Mazzotta C, Traversi C, Caporossi T (2006) Parasurgical therapy for keratoconus by riboflavin–ultraviolet type A rays induced cross-linking of corneal collagen: preliminary refractive results in an Italian study. J Cataract Refract Surg 32:837–845. https://doi.org/10.1016/j.jcrs.2006.01.091
21. Grewal DS, Brar GS, Jain R, Sood V, Singla M, Grewal SP (2009) Corneal collagen crosslinking using riboflavin and ultraviolet-A light for keratoconus: one-year analysis using Scheimpflug imaging. J Cataract Refract Surg 35:425–432. https://doi.org/10.1016/j.jcrs.2008.11.046
22. Zamora KV, Males JJ (2009) Polymicrobial keratitis after a collagen cross-linking procedure with postoperative use of a contact lens: a case report. Cornea 28:474–476. https://doi.org/10.1097/ico.0b013e31818d381a
23. Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C, Caporossi A (2007) Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: in vivo confocal microscopic evaluation. Clin Exp Ophthalmol 35:580–582. https://doi.org/10.1111/j.1442-9071.2007.01536.x
24. Eberwein P, Auw-Hadrich C, Birnbaum F, Maier PC, Reinhard T (2008) Corneal melting after cross-linking and deep lamellar keratoplasty in a keratoconus patient. Klin Monatsblatter Augenheilkd 225:96–98
25. Shalchi Z, Wang X, Nanavaty MA (2015) Safety and efficacy of epithelium removal and transepithelial corneal collagen crosslinking for keratoconus. Eye 29:15. https://doi.org/10.1038/eye.2014.230
26. Bottos KM, Schor P, Dreyfuss JL, Nader HB, Chamon W (2011) Effect of corneal epithelium on ultraviolet-A and riboflavin absorption. Arq Bras Oftalmol 74:348–351. https://doi.org/10.1590/s0004-27492011000500008
27. Bikbova G, Bikbov M (2014) Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin. Acta Ophthalmol. https://doi.org/10.1111/aos.12235
28. Vinciguerra R, Legrottaglie EF, Rosetta P, Azzolini C, Vinciguerra P (2014) Iontophoresis corneal collagen cross linking for progressive keratoconus. Invest Ophthalmol Vis Sci 55:4212
29. Li N, Fan Z, Peng X, Pang X, Tian C (2016) Clinical observation of transepithelial corneal collagen cross-linking by iontophoresis of riboflavin in treatment of keratoconus. Eye Sci 29:160–164

30. Lombardo M, Serrao S, Raffa P, Rosati M, Lombardo G (2016) Novel technique of transepithelial corneal crosslinking using iontophoresis in progressive keratoconus. J Ophthalmol. https://doi.org/10.1155/2016/7472542
31. Vinciguerra P, Romano V, Rosetta P, Legrottaglie EF, Piscopo R, Fabiani C, Azzolini C, Vinciguerra R (2016) Transepithelial iontophoresis versus standard corneal collagen cross-linking: 1-year results of a prospective clinical study. J Refract Surg 32:672–678. https://doi.org/10.3928/1081597x-20160629-02
32. Jia H-Z, Pang X, Fan Z-J, Peng X-J (2017) Iontophoresis-assisted versus standard corneal crosslinking for progressive keratoconus. Ann Eye Sci 2:9. https://doi.org/10.21037/aes.2017.01.01
33. Bikbova G, Bikbov M (2016) Standard corneal collagen crosslinking versus transepithelial iontophoresis-assisted corneal crosslinking, 24 months follow-up: randomized control trial. Acta Ophthalmol 94(7):e600–e606. https://doi.org/10.1111/aos.13032