



Comparison of the Depth of the Stromal Demarcation Line and Clinical Outcomes in Corneal Crosslinking Treatment with Hydroxypropyl Methylcellulose-Based and Vitamin E-TPGS-Based Riboflavin Solutions

Hafize Gokben Ulutas,¹ Ayse Balicki Tufekci²

¹Department of Ophthalmology, University of Health Sciences, Bursa City Hospital, Bursa, Türkiye

²Department of Ophthalmology, University of Health Sciences, Ankara Training and Research Hospital, Ankara, Türkiye

Abstract

Objectives: This study aims to compare the visual and topographic results and the depth of demarcation line in patients who underwent epithelial-off-cross-linking (CXL) with riboflavin solutions containing D alpha-tocopheryl polyethylene-glycol 1000 succinate (Vitamin E-TPGS) and 1.1% hydroxypropyl methylcellulose (HPMC).

Methods: Patients with progressive keratoconus, 26 treated with HPMC (Group 1) and 34 treated with Vitamin E-TPGS (Group 2), were evaluated retrospectively. Best corrected visual acuity, spherical equivalent, refractive cylinder, and corneal topography parameters (keratometry of flat and steep meridians (K1 and K2), maximum keratometry (Kmax), central, thinnest, and apical corneal thickness) at baseline and post-operative follow-up visits were compared (1, 3, 6, and 12 months). Corneal stromal demarcation line depth (DLD) was measured centrally, nasally, and temporally (1.5 mm and 3 mm distance from the center of the cornea) by anterior segment optical coherence tomography at 1 month post-operatively, and changes in endothelial cell density (ECD) at 6 months were compared between the two groups.

Results: There was no difference between the two groups in terms of age and gender. The mean DLD was deeper in group 1 ($325.95 \pm 76.55 \mu\text{m}$) than in group 2 ($267.94 \pm 54.2 \mu\text{m}$) ($p=0.002$). In the 3rd month, K1, K2, and Kmax changes, while a decrease was observed in Group 1 compared to the baseline; an increase was observed in Group 2 (all $p<0.05$). At the end of the 1 year, K1, K2, and Kmax were decreased in both groups. There was no difference in ECD change between baseline and 6 months in both riboflavin solutions ($p=0.12$).

Conclusion: HPMC-based riboflavin appears to be associated with a deeper demarcation line than Vitamin E-TPGS-based riboflavin. While a decrease in K values was observed in the earlier period in HPMC, no difference was observed between Vitamin E-TPGS and HPMC at the 12th month in terms of efficacy. Both riboflavin solutions were safe for the endothelium.

Keywords: Crosslinking, demarcation line, keratoconus, vitamin E TPGS-based riboflavin

Introduction

Keratoconus is usually chronic and progressive cornea thinning and steepening due to the stroma's biomechanical instability. It is manifested by irregular astigmatism and de-

creased visual acuity (1). It usually begins in the 2nd and 3rd decades of life. Corneal involvement often has a bilateral and asymmetrical clinical manifestation (2). Corneal cross-linking (CXL) treatment was effective in arresting progression in the

How to cite this article: Ulutas HG, Balicki Tufekci A. Comparison of the depth of the stromal demarcation line and clinical outcomes in corneal crosslinking treatment with hydroxypropyl methylcellulose-based and vitamin E-TPGS-based riboflavin solutions. *Beyoglu Eye J* 2025; 10(3): 159-167.

Address for correspondence: Hafize Gokben Ulutas, MD. Department of Ophthalmology, University of Health Sciences, Bursa City Hospital, Bursa, Türkiye

Phone: +90 224 295 50 00 **E-mail:** gokbenbilekulutas@gmail.com

Submitted Date: October 16, 2024 **Revised Date:** April 17, 2025 **Accepted Date:** June 17, 2025 **Available Online Date:** September 25, 2025

Beyoglu Eye Training and Research Hospital - Available online at www.beyoglu-eye.com

Copyright © Author(s) This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).



early stages of the disease in an animal experimental study conducted in 1997 (3). CXL treatment, which increases the biomechanical and biochemical stiffness of the cornea, was first applied to human patients in 2003 (4). The conventional CXL technique (CCXL), also known as the Dresden protocol, is applied with ultraviolet-A (UVA) irradiation (370 nm light at 3 mW/cm²) for 30 min after epithelial peeling and instillation of 0.1% riboflavin in 20% dextran solution (vitamin B2). The UV ray diameter is 7 mm, and the total energy applied is 5.4 J/cm² (5). After demonstrating the effectiveness of the Dresden protocol, various techniques were developed to increase the safety and effectiveness of CXL and shorten the treatment time, such as accelerated and customized technics. The accelerated CXL (ACXL) method, which transmits high UVA radiation in a shorter time, the transepithelial epi-on CXL method applied with chemical enhancers, and the iontophoresis CXL method, in which the penetration of the riboflavin solution is increased by using a small electric current, are examples of commonly used methods (6).

A wide variety of riboflavin formulations are currently available on the market. They contain additional components designed to increase the riboflavin penetration into the stroma (6). The osmolarity of riboflavin solutions affects the corneal thickness and is the reason for preference in achieving safe corneal thickness. In recent years, riboflavin solutions containing D alpha-tocopheryl polyethylene-glycol 1000 succinate (Vitamin E-TPGS) based riboflavin has been used in the market. It has been shown that Vitamin E-TPGS increases riboflavin permeability and also provides photoprotection against free radicals formed during photo-induced processes (7).

UVA light energy is known to be effective in corneal regions where riboflavin is absorbed. The cross-linking effect was more effective in the anterior half of the stroma due to the reduction in UVA radiation throughout the depth of the corneal stroma (8). It is said that changes in riboflavin application time and concentration have little effect on the depth of corneal stromal penetration (9). Seiler et al. (10) suggested the demarcation line as an indicator of the depth and efficacy of CXL treatment. Some studies state that the effectiveness of CXL is related to the depth of the demarcation line (11,12). Different results were observed when the demarcation line depths (DLDs) formed with different CXL protocols were compared (12-15).

In this study, we aimed to compare the visual and topographic results, DLD, and endothelial cell density (ECD) after epi-off ACXL with riboflavin solutions containing Vitamin E-TPGS and 1.1% hydroxypropyl methylcellulose (HPMC).

Methods

This retrospective study was conducted in a tertiary hospital's Cornea unit of the Ophthalmology department. Patients who received CXL treatment for progressive keratoconus between January 2020 and January 2022 were included in the study. The hospital ethics committee approved the study (2011-KAEK-25 2022/06-15), and the authors adhered to the principles of the Declaration of Helsinki in all protocols. Corneal topographic (Sirius Topography, CSO, Florence, Italy) data, together with the presence of irregular myopic astigmatism and biomicroscopic examination findings (central or paracentral steepening, Fleischer ring, apical scarring, and Vogt lines) were used for the diagnosis of keratoconus. Progressive keratoconus was defined by the presence of one of the following criteria within the past 1 year (16). An increase of more than 1.0 D in astigmatism, a decrease in corneal thickness of more than 25 microns, an increase of more than 1.0 D in the steepest corneal axis, and a decrease in visual acuity by one line on the Snellen chart. Patients with the following criteria were excluded from the study: The thinnest corneal thickness on topography is <370 microns, presence of corneal scar or opacity, history of infective keratitis, pregnancy or lactation period, presence of chronic, topical, and systemic drug use and presence of autoimmune and connective tissue disease. In addition, patients with a post-operative follow-up period of <1 year and patients with significant post-operative corneal haze were not included in the study.

A complete ophthalmologic examination, including best corrected visual acuity, spherical equivalent, cylindrical value, intraocular pressure, biomicroscopic, and fundoscopic examination of the patients included in the study, pre-operative, post-operative 1 month, 3 months, 6 months, and 12 months were recorded. Flat (K1), steep (K2), and maximum keratometry (Kmax) values and thinnest (TCT), apex (ACT), and central corneal (CCT) thickness values were recorded from the corneal topography parameters evaluated in all examinations of the patients. In addition, corneal ECD was recorded by specular microscopy (NSP-9900, NonconRobo, Konan, Japan) pre-operatively and 6 months post-operatively. One month after the CXL procedure, corneal crossline images obtained with the anterior segment module of Optovue optic coherence tomography (iVue; Optovue Inc., Fremont, CA, USA) were examined. The distance measured between the outer surface of the corneal epithelium and the hyper-reflective line in the stroma using the caliper instrument of the device was defined as the DLD. DLD was measured from the cornea's center and the nasal and temporal regions at 1.5- and 3-mm distances from the center. The average DLD value of the five measured points was recorded. Figures 1 and 2 show DLD measurements on anterior segment optical coherence tomography (OCT) of both groups.

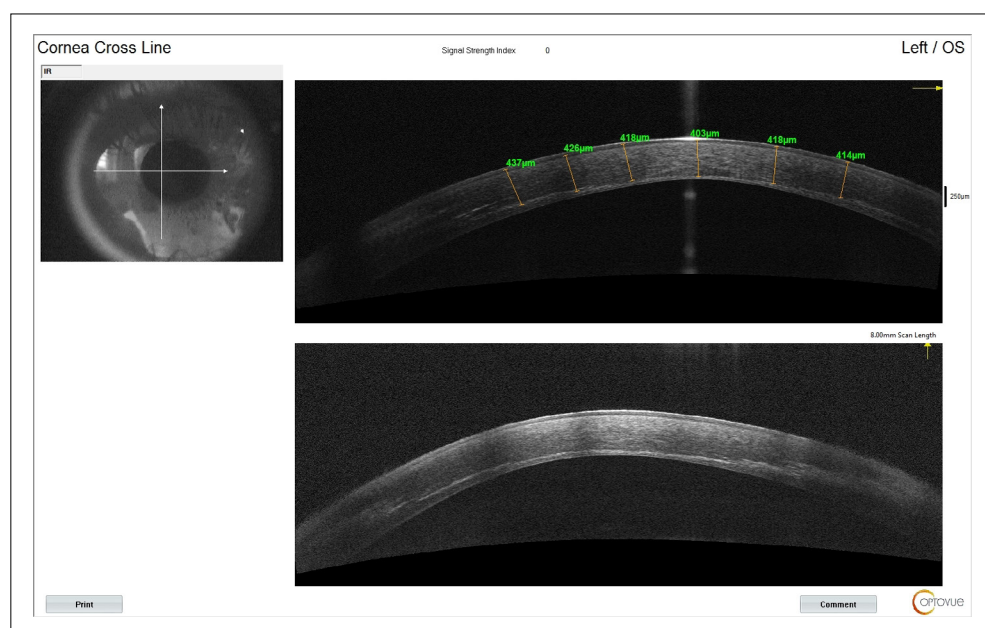


Figure 1. Demarcation line depth measurements of patients in the hydroxypropyl methylcellulose group at the 1st post-operative month.

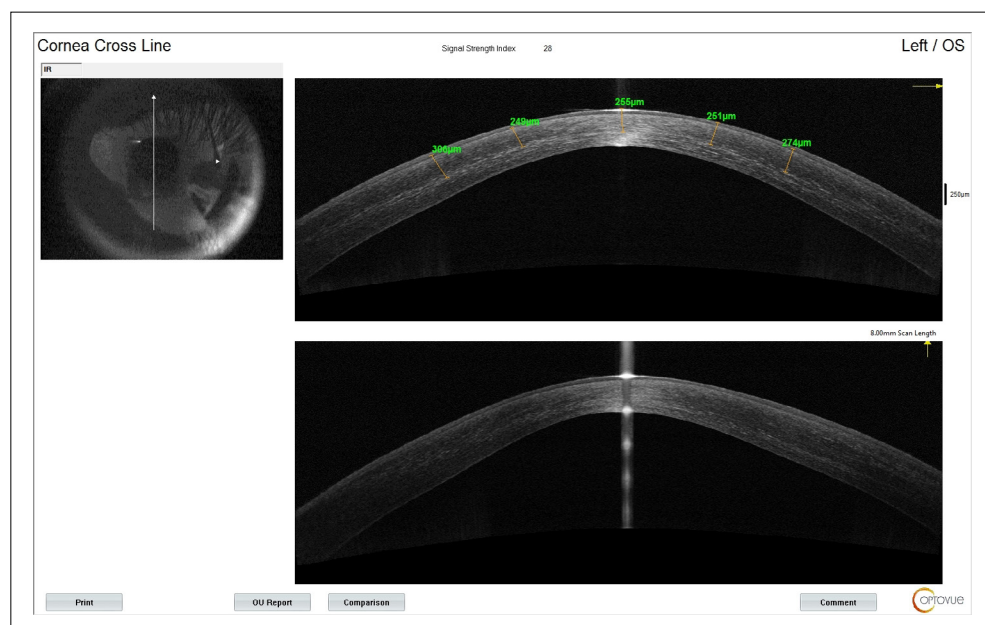


Figure 2. Demarcation line depth measurements of patients in the Vitamin E TPGS group at the 1st post-operative month.

In this retrospective study, patients who had CXL with two different riboflavin solutions available in the hospital pharmacy at different times were divided into two groups. There was no specific feature in choosing riboflavin for the patients. Group 1 consisted of patients treated with riboflavin with HPMC (MedioCROSS® M Avedro 0.1% riboflavin, HPMC 1.1%) and Group 2 with riboflavin with Vitamin E-TPGS (Ribofast® Iromed 0.1% riboflavin, Vitamin E-TPGS).

Pre-operative and post-operative changes in both groups' ophthalmological examination findings and corneal topographic data were compared. In addition, the changes in corneal ECD and DLD values were compared. The correlation between DLD and the change in post-operative values was evaluated.

Surgical Technique

After topical anesthetic drops, 10% diluted ethyl alcohol was applied to the corneal epithelial surface for 15 s with a

9 mm ring. The corneal epithelium was debrided. Patients treated with 1.1% HPMC and 0.1% isotonic riboflavin were instilled every 2 min for 30 min. A 0.1% isotonic riboflavin drop with Vitamin E-TPGS was instilled every 30 s for 15 min. The UVA device (Peschke trade CCL-Vario Cross-linking system, Switzerland) was adjusted to be at an appropriate distance from the eye, and the beam was focused on the corneal center. Nine mW/cm² of 370 nm UVA (total 5.4 J/cm²) was applied to all cases for 10 min as ACXL.

After the cornea was rinsed with sterile saline, a drop of moxifloxacin hydrochloride (0.5%; Vigamox, Alcon Laboratories, Fort Worth, TX) was instilled, and a soft contact lens was inserted. As a post-operative treatment, topical antibiotics (0.5% moxifloxacin hydrochloride) and artificial tears were given to all patients 5 times a day on the 1st day. On the 2nd day, a topical steroid (0.1% dexamethasone) was added to the treatment. If the cornea was completely epithelialized on the 3rd or 4th day, the bandage contact lens was removed. After the 2nd week, only artificial tears and topical loteprednol drops were applied 3 times a day for 1 month. All patients were examined on the 1st day, 1st week, 1st, 3rd, 6th, and 12th months after the procedure.

Statistical Analysis

Power analysis was performed using the G*Power 3.1.9.6 program to calculate the sample size. When the effect size of the difference between the groups in terms of DLD measurement was determined as 0.479, with a power level of 80% and a significance level of 5%, the minimum number of patients to be included in the study in each group was determined as 26.

The data were examined by the Shapiro–Wilk test to determine whether or not it presents a normal distribution. The results were presented as mean±standard deviation, median (minimum–maximum), or frequency and percentage. Normally distributed data were compared with independent samples t-tests, and Mann-Whitney U tests were used for non-normally distributed data. Repeated measurements were compared between groups by calculating the difference (Change=last measure first measure) according to the baseline measurement. Categorical variables were compared using Pearson's Chi-square, Fisher's exact, and Fisher-Freeman-Halton tests between groups. The Pearson correlation coefficient was calculated for the relationship between variables. Statistically, the significance level was accepted as $\alpha=0.05$. Statistical analyses were performed with the IBM Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.).

Results

Sixty eyes of 60 progressive keratoconus patients, 24 females, and 36 males, were included in this study. The patients were divided into two groups according to the riboflavin solutions used during ACXL. Group 1 consisted of 26 patients treated with HPMC-based riboflavin solution, and Group 2 consisted of 34 patients treated with CXL with Vitamin E-TPGS-based riboflavin solution. There was no statistically significant difference between the two groups in the patient's demographic characteristics, pre-operative ophthalmological examination, and topographic parameters. Demographic data and pre-operative examination findings of the patients are summarized in Table 1. While the median Kmax value decreased by -0.03 ± 1.52 D in Group 1 in the post-operative 3rd month, it increased by 1.18 ± 1.62 D in Group 2. The difference is statistically significant ($p=0.01$). Mean Kmax changes at 1 year post-operatively were -0.45 ± 1.33 D and -0.44 ± 1.53 D in Group 1 and Group 2, respectively, and there was no difference between the two groups ($p<0.05$). Table 2 shows the changes in keratometry values from the baseline at the post-operative 1st, 3rd, 6th, and 12th months.

Mean DLD measurements were statistically higher ($p=0.002$) in the HPMC group compared to the HPMC and Vitamin E-TPGS groups (325.95 ± 76.5 and 267.94 ± 54.2 μm , respectively). The value of the DLD in all regions in the post-operative 1st month of both groups is shown in Table 3.

In the correlation analysis of DLD and keratometry values, there was a weak positive correlation between the DLD and the change in Kmax value at 6 months ($r=0.371$, $p=0.009$). No significant correlation was detected in all any of the other parameters shown in Table 4.

Discussion

The effectiveness and safety of the CCXL procedure have been proven in many studies since 2003. Riboflavin is absorbed by corneal tissue through drops applied every 2 min for 30 min. UVA radiation is irradiated at three mW/cm² for 30 min (5.4 J/cm² energy dose). Free oxygen radicals formed as a result of the interaction of riboflavin molecules absorbed in the tissue and UVA light rays strengthen corneal biomechanics by creating covalent bonds within and between collagen fibers. Over the years, different techniques have been tried to shorten the surgery time without reducing the effect of CXL. With the ACXL protocol, successful results can be achieved in strengthening the corneal tissue by exposing it to higher-intensity UVA in a shorter time (17,18). Shortening the surgery time may reduce corneal dehydration and cause fewer complications, such as infection. Riboflavin solutions containing Vitamin E TPGS have recently been commercially

Table 1. Comparison of demographic data and baseline findings of both groups

Parameters	Group 1 (HPMC)	Group 2 (Vitamin E-TPGS)	p*
	n=26	n=34	
Age (years), Mean±SD	23.37±7.13	23.97±6.79	0.739
Gender M:F	12:14	22:12	0.151
BCVA	0.51±0.27 (0.05–1.0)	0.51±0.27 (0.05–1.0)	0.747
SE (D)	−4.12±2.71 (−0.5–11.0)	−4.64±2.7 (1.0–13.0)	0.341
Cylinder (D)	−3.28±−2.29 (−0.27–5.5)	−2.75±1.48 (−0.18–5.5)	0.469
Kmax (D)	53.47±4.91 (46.59–64.20)	54.64±5.33 (44.84–75.53)	0.383
K1 (D)	45.04±2.25 (41.56–50.82)	44.98±2.91 (40.73–55.92)	0.727
K2 (D)	48.12±2.27 (43.99–52.12)	47.73±3.33 (43.25–58.90)	0.254
TCT	462.85±38.97 (383–546)	455±49.50 (369–567)	0.273
ACT	478.93±40.25 (391–565)	470.30±49.20 (370–579)	0.259
CCT	476.19±40.91 (400–569)	467.12±52.34 (371–576)	0.466
ECD	2563.64±339 (1721–2907)	2855±309.71 (2119–3401)	0.018

SD: Standard deviation; HPMC: Hydroxypropyl methylcellulose; Vitamin E-TPGS: D alphanatocopheryl polyethylene-glycol 1000 succinate; M: Male; F: Female; BCVA: Best corrected visual acuity; SE: Spherical equivalent; Kmax: Maximum keratometry; K1: Flat keratometry; K2: Steep keratometry; TCT: Thinnest corneal thickness; ACT: Apical corneal thickness; CCT: Central corneal thickness; ECD: Endothelial cell density.

Table 2. Comparison of changes in Keratometric values in the 1st, 3rd, 6th, and 12th months post-operatively compared to the baseline

Variables	Group 1 (HPMC)		Group 2 (Vitamin E-TPGS)		p
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	
Change in Kmax					
1 month	1.10±2.01	0.86 (−1.88–7.52)	1.26±1.83	1.48 (−3.15–4.8)	0.757
3 months	−0.03±1.52	−0.24 (−2.58–3.08)	1.18±1.62	1.15 (−1.54–5.75)	0.010
6 months	0.02±1.42	−0.19 (−2.48–2.89)	−0.33±1.99	−0.03 (−5.5–3.50)	0.492
12 months	−0.45±1.33	−0.59 (−3.83–2.26)	−0.44±1.53	−0.52 (−3.42–2.67)	0.973
Change in K1					
1 month	0.39±1.01	0.22 (−1.17–4.44)	0.41±0.68	0.45 (−0.91–2.57)	0.368
3 months	−0.26±0.48	−0.24 (−1.12–0.57)	0.18±0.59	0.29 (−1.04–2.20)	0.006
6 months	−0.21±0.62	−0.26 (−1.31–1.96)	−0.38±0.55	−0.41 (−1.68–0.33)	0.717
12 months	−0.61±0.52	−0.46 (−1.31–1.96)	0.60±0.91	−0.62 (−3.61–1.13)	0.831
Change in K2					
1 month	0.34±0.89	0.48 (−2.06–2.06)	0.79±0.91	0.77 (−0.71–4.12)	0.064
3 months	−0.20±0.62	−0.16 (−1.20–1.44)	0.22±0.78	0.18 (−1.26–3.07)	0.022
6 months	−0.26±0.71	−0.22 (−1.48–1.82)	−0.29±0.67	−0.21 (−2.02–0.65)	0.841
12 months	−0.58±0.82	−0.57 (−2.96–0.75)	−0.50±1.01	−0.48 (−3.47–1.96)	0.708

SD: Standard deviation, HPMC: Hydroxypropyl methylcellulose, Vitamin E-TPGS: D alphanatocopheryl polyethylene-glycol 1000 succinate, BCVA: Best corrected visual acuity, Kmax: Maximum keratometry, K1: Flat keratometry, K2: Steep keratometry, TCT: Thinnest corneal thickness, ACT: Apical corneal thickness, CCT: Central corneal thickness.

Table 3. The values of the demarcation line depth in all regions in both groups at post-operative 1st month

	Group 1 (HPMC)	Group 2 (Vitamin E-TPGS)	p
DLD nasal 1	351.92±73.6	292.58±71.4	0.002
DLD nasal 2	336.29±85.6	270.81±58.6	0.002
DLD central	335.22±83.4	267.02±55.7	<0.001
DLD temporal 1	328.66±93.4	259.93±59.1	0.002
DLD temporal 2	322.58±89.4	260.48±67.7	0.004
DLD mean	325.95±76.5	267.94±54.2	0.002
Change in ECD 1 month	-115.25±297.12	-348.66±374.87	0.127

HPMC: Hydroxypropyl methylcellulose; Vitamin E-TPGS: D alphatocopheryl polyethylene-glycol 1000 succinate; DLD: Demarcation line depth; ECD: Endothelial cell density.

Table 4. Correlation between demarcation line depth and keratometry values of all patients

	DLD nasal		DLD central		DLD temporal	
	r	p	r	p	r	p
Change in Kmax 1 month	0.110	0.420	0.167	0.214	0.113	0.406
Change in Kmax 3 month	0.037	0.799	0.055	0.702	0.030	0.838
Change in Kmax 6 month	0.371	0.009	0.349	0.014	0.355	0.013
Change in Kmax 12 month	0.172	0.252	0.172	0.248	0.133	0.378
Change in K1 1 month	0.176	0.195	0.193	0.151	0.174	0.199
Change in K1 3 month	-0.046	0.754	-0.116	0.421	-0.106	0.469
Change in K1 6 month	0.042	0.776	0.067	0.649	0.113	0.443
Change in K1 12 month	0.164	0.277	0.081	0.590	0.147	0.330
Change in K2 1 month	-0.126	0.353	-0.098	0.467	-0.102	0.453
Change in K2 3 month	-0.026	0.858	-0.042	0.771	-0.057	0.695
Change in K2 6 month	0.163	0.268	0.263	0.068	0.311	0.031
Change in K2 12 month	0.165	0.273	0.161	0.281	0.189	0.208

DLD: Demarcation line depth; Kmax: Maximum keratometry; K1: Flat keratometry; K2: Steep keratometry.

available to increase riboflavin penetration into the cornea. The protocol for saturating Vitamin E TPGS-based solutions onto the cornea is to instill them every 30 s for 15 min. Vitamin E TPGS accelerates the stromal penetration of riboflavin, so shorter application times are sufficient, and the solution needs to be instilled at more frequent intervals due to its low osmolarity (19).

ACXL protocol using Vitamin E TPGS-based riboflavin solution significantly shortens the surgery time. A few studies in the literature compare the effectiveness of riboflavin solutions containing Vitamin E TPGS. In the study of Carusa et al., (19) which used Vitamin E TPGS-based riboflavin, the CCXL protocol was compared with a customized accelerated CXL (CFXL) protocol. No difference between the two

protocols was detected in the biomechanical parameters, refractive, and topographic results of the cornea. In the CFXL group performed using the epi-on technique, the Kmax value decreased by -0.99 ± 0.34 D at the 12th month compared to the baseline. In the present study, the Kmax value decreased by -0.44 ± 1.53 D in the 12th month in the group that received epi-off ACXL with Vitamin E TPGS. Crosslinking performed with Vitamin E TPGS-based riboflavin solution also appears effective in halting keratoconus progression with epi-on and epi-off techniques. Rupano et al. (20) compared patients who underwent CCXL using HPMC and dextran-based riboflavin solutions. Kmax change in the HPMC group compared to the baseline values at 1st, 6th, and 12th months were found to be 3.32 ± 4.89 , -0.20 ± 2.22 , and -0.45 ± 2.35 D, respectively. In

the present study, the change in Kmax values in the HPMC group from baseline to the 1st, 6th, and 12th months were found to be 1.10 ± 2.01 , 0.02 ± 1.42 , -0.45 ± 1.33 D, respectively. The 12th-month results were similar, even though they were conducted with CCXL and ACXL methods.

In our study, when we compared the refractive and topographic results of the Vitamin E TPGS and HPMC groups after CXL, best corrected visual acuity (BCVA) was higher in the Vitamin E TPGS group in the 1st and 3rd months than in the HPMC group. However, the change in Kmax values in the 3rd month was significantly lower in the HPMC group (-0.03 ± 1.52 D in the HPMC group vs. Vitamin E TPGS group 1.18 ± 1.62 D, $p=0.010$). Considering the amount of change in the values in the 12th month, similar results were obtained as Kmax -0.45 ± 1.33 D in the HPMC group and -0.44 ± 1.53 D in the Vitamin E TPGS group ($p=0.973$). It can be said that CXL treatment with Vitamin E TPGS-based riboflavin solutions shortens the duration of surgery and is as effective as HPMC-based riboflavin solution, even if an increase in K values is observed without a decrease in BCVA in the early post-operative period.

The demarcation line detected after CXL represents the transition zone between anterior stromal keratocytes' apoptosis with UVA rays and unaffected keratocytes in the posterior stroma. Determination of the demarcation line is important in terms of maintaining endothelial safety, especially in eyes with thin corneas (21). In this study, the depth of demarcation line detected at the post-operative 1st month was 325.95 ± 76.5 μ m in the HPMC-based riboflavin group and 267.94 ± 54.2 μ m in the Vitamin E TPGS-based riboflavin group. Both riboflavin solutions were safe for the endothelium.

In the literature, different results have been shown in studies evaluating DLDs after ACXL and CCXL methods. Hagem et al. (22) found the DLD in the OCT evaluation as 296 ± 54 μ m in the CCXL group and 160 ± 41 μ m in the ACXL group. They did not detect any difference in Kmax and visual acuity values at the end of the 12th month. Brittingham et al. (23) said the ACXL protocol reduces the visibility and depth of the demarcation line and negatively affects topographic results. The DLD has been proposed as an objective marker to determine the effectiveness of treatment. Kyminious et al. (15) found DLD to be 350.78 ± 49.34 μ m in the CCXL group and 288.46 ± 42.37 μ m in the ACXL group ($p=0.005$). They argued that DLD could be an indicator of treatment effectiveness. Unlike the studies mentioned above, Spadeo et al. (24) found similar DLD values in ACXL and CCXL protocols.

In studies comparing HPMC and dextran-based riboflavin solutions, deeper DLD was found in corneas using HPMC riboflavin (25,26). Malhotra et al. (25) found the DLD to be 308.22 ± 84.19 μ m in the HPMC group and 235.33 ± 64.87 μ m

in the dextran group. They thought that both riboflavin solutions were safe for the endothelium. In the study of Özek et al., (27) DDL was compared in CXL performed with hypotonic and dextran-free isotonic riboflavin. DLD was found to be 180.32 ± 10.26 μ m in the hypotonic riboflavin group and 287.21 ± 15.01 μ m in the isotonic riboflavin group ($p<0.05$). They conclude that the use of hypotonic riboflavin causes swelling of the cornea and causes more superficial DLD after CXL. Balıkcı et al. (28) evaluated pachymetry values before and after the instillation of Vitamin E TPGS and 1.1% HPMC-based riboflavin solutions to evaluate the increase in corneal stromal thickness during CXL. They argued that Vitamin E TPGS, used to increase the penetration of riboflavin into the corneal tissue during CXL treatment, significantly increases the corneal thickness compared to HPMC and creates a safer tissue thickness for UVA. In the present study, it was observed that a more superficial demarcation line was formed in epi-off CXL performed with Vitamin E TPGS-based riboflavin solution. Hypotonic riboflavin solutions swell the cornea and create a more superficial DLD. Vitamin E TPGS-based riboflavin solution applied with the epi-off method creates a more superficial DLD by swelling the corneal stroma, such as hypotonic riboflavin. Vitamin E TPGS-based riboflavin solution can be preferred more safely than HPMC in thin corneas because its treatment effectiveness is similar, the depth of the demarcation line is superficial, and the corneal thickness increases significantly during the procedure. The limitations of this study are its retrospective design, short-term follow-up, random selection of patients into groups, and small number of patients.

Conclusion

This study is the first in the literature to evaluate DLD in CXL performed with Vitamin E TPGS-based riboflavin. Both riboflavin solutions were observed to be effective in halting keratoconus progression and safe for endothelial cells in the 1st-year results. Similar clinical results were obtained with the Vitamin E TPGS-based riboflavin solution, although with a more superficial DLD. There is still no consensus in the literature as to whether DLD is an indicator of treatment effectiveness. Different results have been shown in studies. In this study, no significant relationship was found between DLD and treatment effectiveness. Long-term studies with a larger number of patients and a prospective design are needed for definitive evidence.

Disclosures

Ethics Committee Approval: This study was approved by the SBÜ Bursa Hospital Ethics Committee (Date: 06,15,2022, Number: 2011-KAEK-25) and conducted in accordance with the tenets of the Declaration of Helsinki.

Conflict of Interest: None declared.

Funding: The authors declare that this study has received no financial support.

Use of AI for Writing Assistance: Not declared.

Author Contributions: Concept – H.G.U., A.T.B.; Design – H.G.U.; Supervision – H.G.U.; Resource – H.G.U., A.T.B.; Materials – H.G.U., A.T.B.; Data Collection and/or Processing – H.G.U.; Analysis and/or Interpretation – H.G.U.; Literature Search – H.G.U.; Writing – H.G.U.; Critical Reviews – A.T.B.

Acknowledgements: The authors would like to thank biostatistician Prof Dr Güven Özkaya for the statistical analysis of this article. This study was presented in the TOD March Symposium, 2024.

Peer-review: Externally peer-reviewed.

References

- Ferrari G, Rama P. The keratoconus enigma: A review with emphasis on pathogenesis. *Ocul Surf* 2020;18:363–73. [CrossRef]
- Flockerzi E, Xanthopoulou K, Goebels SC, Zemova E, Razafimino S, Hamon L, et al. Keratoconus staging by decades: A baseline ABCD classification of 1000 patients in the Homburg Keratoconus Center. *Br J Ophthalmol* 2021;105:1069–75. [CrossRef]
- Spörl E, Huhle M, Kasper M, Seiler T. Increased rigidity of the cornea caused by intrastromal cross-linking. *Ophthalmologe* 1997;94:902–6. [Article in German] [CrossRef]
- Belin MW, Lim L, Rajpal RK, Hafezi F, Gomes JAP, Cochener B. Corneal cross-linking: Current USA status: Report from the Cornea Society. *Cornea* 2018;37:1218–25. Erratum in: *Cornea*. 2019;38:e49. [CrossRef]
- Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol* 2003;135:620–7. [CrossRef]
- Subasinghe SK, Ogbuehi KC, Dias GJ. Current perspectives on corneal collagen crosslinking (CXL). *Graefes Arch Clin Exp Ophthalmol* 2018;256:1363–84. [CrossRef]
- Ostacolo C, Caruso C, Tronino D, Troisi S, Laneri S, Pacente L, et al. Enhancement of corneal permeation of riboflavin-5'-phosphate through vitamin E TPGS: A promising approach in corneal trans-epithelial cross linking treatment. *Int J Pharm* 2013;440:148–53. [CrossRef]
- Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen fiber diameter in the rabbit cornea after collagen crosslinking by riboflavin/UVA. *Cornea* 2004;23:503–7. [CrossRef]
- Søndergaard AP, Hjortdal J, Breitenbach T, Ivarsen A. Corneal distribution of riboflavin prior to collagen cross-linking. *Curr Eye Res* 2010;35:116–21. Erratum in: *Curr Eye Res* 2010;35:1044. [CrossRef]
- Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. *Cornea* 2006;25:1057–9. [CrossRef]
- Moramarco A, Iovieno A, Sartori A, Fontana L. Corneal stromal demarcation line after accelerated crosslinking using continuous and pulsed light. *J Cataract Refract Surg* 2015;41:2546–51. [CrossRef]
- Yam JC, Chan CW, Cheng AC. Corneal collagen cross-linking demarcation line depth assessed by Visante OCT After CXL for keratoconus and corneal ectasia. *J Refract Surg* 2012;28:475–81. [CrossRef]
- Kymionis GD, Tsoularas KI, Liakopoulos DA, Skatharoudi CA, Grentzelos MA, Tsakalis NG. Corneal stromal demarcation line depth following standard and a modified high intensity corneal cross-linking protocol. *J Refract Surg* 2016;32:218–22. [CrossRef]
- Kymionis GD, Tsoularas KI, Grentzelos MA, Plaka AD, Mikropoulos DG, Liakopoulos DA, et al. Corneal stroma demarcation line after standard and high-intensity collagen crosslinking determined with anterior segment optical coherence tomography. *J Cataract Refract Surg* 2014;40:736–40. [CrossRef]
- Kymionis GD, Tsoularas KI, Grentzelos MA, Liakopoulos DA, Tsakalis NG, Blazaki SV, et al. Evaluation of corneal stromal demarcation line depth following standard and a modified-accelerated collagen cross-linking protocol. *Am J Ophthalmol* 2014;158:671–5.e1. [CrossRef]
- Santodomingo-Rubido J, Carracedo G, Suzaki A, Villa-Collar C, Vincent SJ, Wolffsohn JS. Keratoconus: An updated review. *Cont Lens Anterior Eye* 2022;45:101559. [CrossRef]
- Shajari M, Kolb CM, Agha B, Steinwender G, Müller M, Herrmann E, Schmack I, et al. Comparison of standard and accelerated corneal cross-linking for the treatment of keratoconus: A meta-analysis. *Acta Ophthalmol* 2019;97:e22–35. [CrossRef]
- Waszczykowska A, Jurowski P. Two-year accelerated corneal cross-linking outcome in patients with progressive keratoconus. *Biomed Res Int* 2015;2015:325157. [CrossRef]
- Caruso C, Epstein RL, Troiano P, Napolitano F, Scarinci F, Costagliola C. Topo-pachimetric accelerated epi-on cross-linking compared to the dresden protocol using riboflavin with vitamin E TPGS: Results of a 2-year randomized study. *J Clin Med* 2021;10:3799. [CrossRef]
- Rapuan PB, Mathews PM, Florakis GJ, Trokel SL, Suh LH. Corneal collagen crosslinking in patients treated with dextran versus isotonic hydroxypropyl methylcellulose (HPMC) riboflavin solution: A retrospective analysis. *Eye Vis (Lond)* 2018;5:23. [CrossRef]
- Mazzotta C, Balestrazzi A, Traversi C, Baiocchi S, Caporossi T, Tommasi C, et al. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: Ultrastructural analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. *Cornea* 2007;26:390–7. [CrossRef]
- Hagem AM, Thorsrud A, Sandvik GF, Råen M, Drolsum L. Collagen crosslinking with conventional and accelerated ultraviolet-A irradiation using riboflavin with hydroxypropyl methylcellulose. *J Cataract Refract Surg* 2017;43:511–7. Erratum in: *J Cataract Refract Surg* 2019;45:390. [CrossRef]

23. Brittingham S, Tappeiner C, Frueh BE. Corneal cross-linking in keratoconus using the standard and rapid treatment protocol: Differences in demarcation line and 12-month outcomes. *Invest Ophthalmol Vis Sci* 2014;55:8371–6. [\[CrossRef\]](#)
24. Spadea L, Di Genova L, Tonti E. Corneal stromal demarcation line after 4 protocols of corneal crosslinking in keratoconus determined with anterior segment optical coherence tomography. *J Cataract Refract Surg* 2018;44:596–602. [\[CrossRef\]](#)
25. Malhotra C, Jain AK, Gupta A, Ram J, Ramatchandirane B, Dhingra D, et al. Demarcation line depth after contact lens-assisted corneal crosslinking for progressive keratoconus: Comparison of dextran-based and hydroxypropyl methylcellulose-based riboflavin solutions. *J Cataract Refract Surg* 2017;43:1263–70. [\[CrossRef\]](#)
26. Chacra LM, Helwe C, Assaf JF, Yehia M, Baroud S, Torres-Netto EA, et al. Accelerated corneal crosslinking with 20'-soaking hydroxypropyl methyl cellulose/riboflavin vs conventional crosslinking with 30'-soaking dextran/riboflavin. *J Cataract Refract Surg* 2024;50:236–43. [\[CrossRef\]](#)
27. Ozek D, Kemer OE, Ozer PA. Corneal stromal depth of the demarcation line in 'accelerated corneal cross-linking' with different concentrations of riboflavin solutions. *Int Ophthalmol* 2019;39:1329–35. [\[CrossRef\]](#)
28. Balıkcı AT, Ulutaş HG. Comparison of pachymetry values during crosslinking with different riboflavin solutions. *Ann Med Res* 2023;30:1483–6. [\[CrossRef\]](#)