

Evaluation of Corneal Stromal Demarcation Line Depth in Epithelium-Off Standard, Epithelium-Off Accelerated and Transepithelial Cross-Linking in Keratectasia

M.A.Soliman, A.M.Saeed and A.M.Naguib

Ophthalmology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt

Email:ahmedshakran@gmail.com

Abstract

Background: Corneal collagen cross-linking (CXL) has been increased to moderate and halt the development of keratoconus and LASIK ectasia as a viable method. This research is intended to research the corneal current demarcation line (DL) and to compare the depth of the Corneal Strom Demarcation Line utilising anterior segment epithelial, epithelial, accelerated, and transepithelial interconnection (AS-OCT). **Methods:** 24 Eyes that match the criteria for inclusion of Cross-Linking patients split evenly into three interventions throughout the last 6 months were included in this research. Topcon 3D spectral domain OCT 2000 was used to measure the demarcation line depth. **Outcomes:** The DL depth indicated an overall significant difference in the conventional epi-off group (340 microns) across analysed groups, which is much greater than the accelerated epi-off (267 microns) and the accelerated epi-on (190 microns). In the accelerated epi-off it was also much greater than the accelerated epi-on. **Conclusion:** stromal demarcation line revealed a larger depth in the standard epidemic than rapid epidemic, which depth is higher than that of transepithelial cross-linking, meaning that standard epidema, accelerated epidemic and transepitude techniques are used to determine a larger depth of treatment.

Keywords: Corneal, cross-linking, CXL, Demarcation Line, Keratectasia.

1. Introduction

Cross-linking of keratoconus and post-LASIK ectasia was developed as the potential technique to control or prevent the advancement of keratoconus. CXL is considered the best technique to stop keratoconus development by increasing corneal rigidity. In early clinical studies, keratoconus advancement was seen in majority of the CXL-treated eyes and regression was seen in 70 percent of the eyes. [1]

Several studies have developed and validated the efficacy and safety of a conventional CXL method, often known as the Dresden protocol.

12-15; The interplay between 0.1 percent riboflavin molecules absorbed into the corn tissue and light UVA rays, irradiated for 30 minutes at 3 mW/cm² (5.4 J/cm² dosage of energy), creates reactive oxygen species that encourage molecular bridges between and among collagen fibres. 16 In recent years, emphasis was focused on the possibilities of designing novel CXL procedures to limit UV exposure length, complication rate, patient pain and potential adverse effects. 17 The accelerated high-flow CXL, based on the photochemical reciprocity principle, commonly known as the Bunsen-Roscoe law, minimises UV exposure and patient discomfort for the length of the CXL operation. 18 A sufficient level of corneal absorption of riboflavin is needed in order to achieve an efficient CXL. The epithelial decline in conventional CXL therefore has the twin aim to overcome the barrier generated by tight intersections of the corneal epithelium that would restrict macromolecule penetration as Vitamin B₂, thereby preventing UV absorption from the epithelium. 19 Various ways have been suggested to improve the penetration of transepithelial riboflavin, such as enhancing imbibition formulations of riboflavin to

increase its absorption. Therefore, numerous topically active pharmacological products including benzalconium chloride and EDTA are utilised to improve epithelial penetration in order to prevent a CXL cytotoxic impact on corneal endothelium, crystalline lens and other intraocular tissues, particularly in ultra-keratoconic corneas (pachymetry of less than 400 mm). [2]

Following CXL, a corneal stroma demarcation line may be distinguished at a depth of about 300 µm during a slitlamp examination 2 weeks after treatment.

An earlier Segment Optical Coherence Tomography (AS-OCT) may also be used to identify a corneal stroma demarcation line following CXL, which might signify the adequacy of CXL therapy. [3] This research is intended to investigate and compare the corneal current demarcation line in Epithelium-Off Standard, Epithelium-Off Accelerated and Transepithelial Cross-Link utilising Ocular Ocular Tomography Anterior Segment (AS-OCT)

2. Patients and methods

Type of research: study of observation

Population study: patients who have cross-linked during the past six months

Our investigation was done in the past six months on individuals who had been cross-linked. 24 eyes have been evenly split among these techniques;

- Standard epi-off (8 eyes).
- Accelerated epi-off (8 eyes).
- Accelerated epi-on (8 eyes).

Inclusion criteria

- Age : 14- 50 years old

Exclusion criteria

- Corneal dystrophies
- Corneal scar
- Intra-Corneal implants

Sample size : 24 Eyes who fit the inclusion criteria divided equally to the 3 procedures.

Study tool: Anterior Segment OCT.

Included Patients will be subjected to full ophthalmologic examination including:

- Unaided Visual Acuity (UAVA) and Best Corrected Visual Acuity (BCVA)
- Slit lamp examination
- Anterior Segment OCT

Standard epi-off eyes after epithelium removal by using a blunt metal spatula to mechanically scrape off a 9.0 mm diameter area, the cornea was soaked for 15 minutes in riboflavin 0.1% solution and then exposed to a solid-state UVA illuminator for 30 minutes, during which the riboflavin solution was applied every 3 minutes. The UVA illuminator was calibrated to 3.0 mW/cm² of surface irradiance (5.4 J/cm² surface dose) using a UV light meter at the specified working distance.

Accelerated epi-off eyes underwent UVA irradiation After epithelium removal by using a blunt metal spatula to mechanically scrape off a 9.0 mm diameter area, the cornea was soaked for 15 minutes in riboflavin 0.1% solution and then exposed to a solid-state UVA illuminator. Then, the corneas were irradiated by the device calibrated to 9 mW/cm² (5.4 J/cm² surface dose) and UVA irradiation was applied on the central 9.0 mm of the cornea for 10 minutes. During this time, the riboflavin solution was applied to the cornea every 3 minutes.

Transepithelial epi-on 3mW/cm² Without any de-epithelialization, riboflavin 0.1% solution in 15% dextran T500 containing sodium ethylenediaminetetraacetic acid (EDTA) 0.01% and trometamol was instilled Then, the corneas were irradiated by the device calibrated to 10 mW/cm² (5.4 J/cm² surface dose) and UVA irradiation was applied

on the central 9.0 mm of the cornea for 9 minutes. During this time, the riboflavin solution was applied to the cornea every 3 minutes.

2.1. Statistical analysis

Data management and statistical analysis were done using SPSS vs.25. (IBM, Armonk, New York, United States). Numerical data were summarized as means and standard deviations. Categorical data were summarized as numbers and percentages. The non-parametric Kruskal Wallis test was used for comparing numerical data due to the small number in each group, which made the normality testing invalid. Categorical data were compared using Fisher’s exact test, if appropriate. All P values were two-sided. P values less than 0.05 were considered significant.

3. Results

There were no significant differences between the studied groups regarding age (P value = 0.365), gender (P value = 0.818), and eye laterality (P value = 0.664). Table (1).

UAVA and BCVA showed an overall significant difference between the study groups; P values were 0.04 and 0.027, respectively. UAVA Post-hoc analysis showed that it was significantly lower in the standard epi-off group (0.13) than the accelerated epi-on group (0.29), with no significant differences between the accelerated epi-off group (0.24) and both the standard epi-off (0.13) and the accelerated epi-on (0.29) groups. BCVA post hoc analysis revealed that it was significantly lower in the standard epi-off group (0.5) than the accelerated epi-off group (0.8), with no significant differences between the accelerated epi-on group (0.7) and both the standard epi-ff (0.5) and the accelerated epi-off (0.8) groups. Figure 1

Table (1) General characteristics of the studied groups

		Standard epi-off (n = 6 cases , 8 eyes)	Accelerated epi-off (n = 6 cases, 8 eyes)	Accelerated epi-on (n = 6 cases, 8 eyes)	P-value
Age (Years)	Mean ±SD	26 ±3	28 ±10	31 ±5	0.365
Gender	Males n (%)	1 (16.7)	2 (33.3)	3 (50.0)	0.818
	Females n (%)	5 (83.3)	4 (66.7)	3 (50.0)	
Eye	OD n (%)	2 (25.0)	4 (50.0)	4 (50.0)	0.664
	OS n (%)	6 (75.0)	4 (50.0)	4 (50.0)	

Kruskal Wallis test was used for age. Fisher’s exact test was used for gender and eye laterality

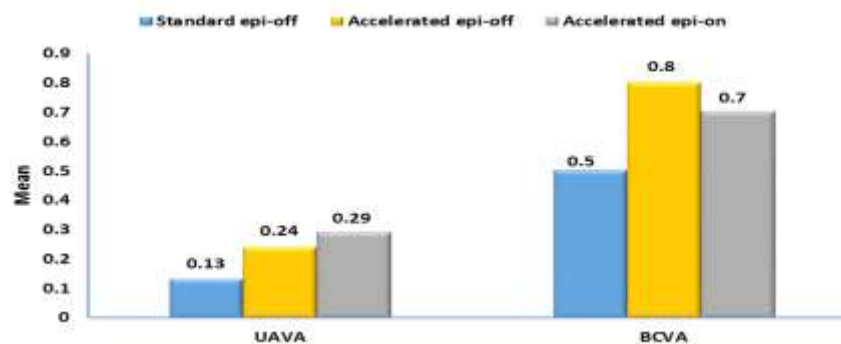


Fig. (1) UAVA and BCVA in the studied groups.

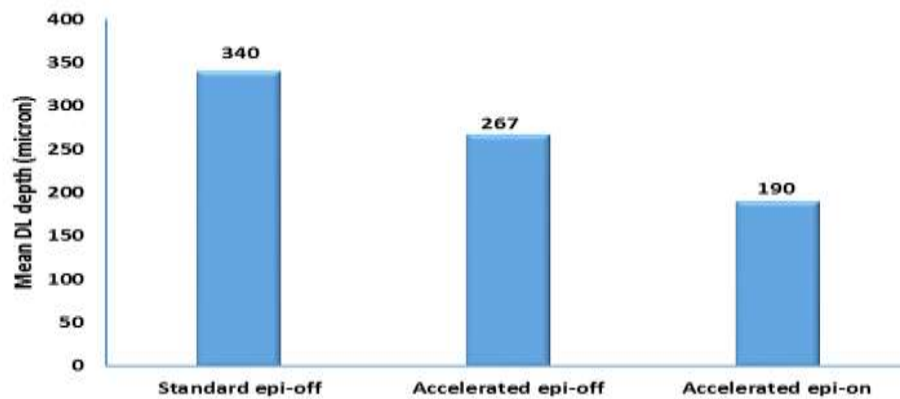


Fig. (2) Demarcation line in the studied groups.

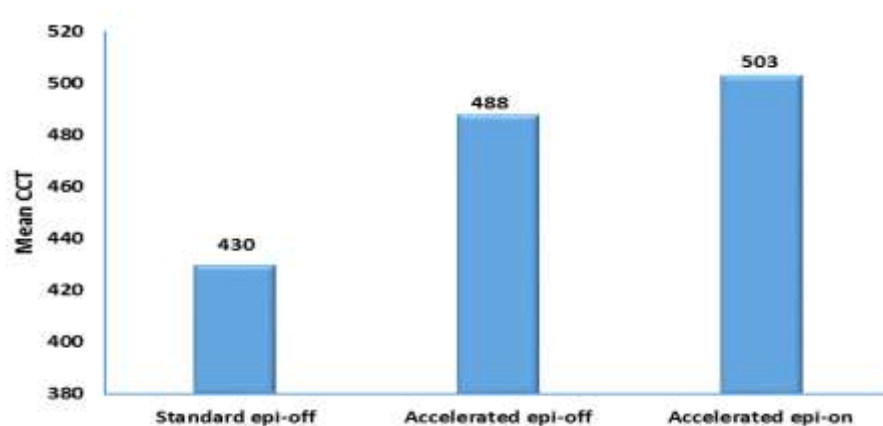


Fig. (3) Central corneal thickness in the studied groups.

DL depth showed an overall significant difference between the studied groups; P value was <0.001. Post hoc analysis revealed that it was significantly higher in the standard epi-off group (340 microns) than the accelerated epi-off (267 microns) and the accelerated epi-on (190 microns) groups. Also, it was significantly higher in the accelerated epi-off than the accelerated epi-on. Figure 2

Central corneal thickness (CCT) showed an overall significant difference between the studied groups; the P-value was 0.002. Post hoc analysis revealed that it was significantly lower in the standard epi-off group (430) than the accelerated epi-off (488) and the accelerated epi-on (503) groups, with no significant difference between the accelerated epi-off and the accelerated epi-on groups. Figure 3

4. Discussion

Our investigation indicated a mean demarcation line depth of 340 ± 27 microns using standard eye-group Epi-Off. Average depth of demarcation was 267 ± 16 microns in the accelerated epi-off group eyes. TransEpithelial group eyes exhibited a mean line demarcation depth of 190 ± 20 microns (P <0.001) based on Topcon 3D OCT2000 AS-OCT measurements.

Overall, the depth of the demarcation line demonstrated a significant difference between the

groups investigated; P was <0.001. Post-hoc analysis showed that the conventional epi-off group (340 mikrons) is much deeper than the accelerated epi-off group (267 microns) and the accelerated epi-on group (190 microns). In the accelerated epi-off it was also much greater than the accelerated epi-on.

These results reflect the results of the Kymionis et al study,[4], 21 mean DL. In nine eyes with normal epi-off procedure (350.78 ± 49.34 mm), the depth was substantially greater than that of 12 eyes treated with the expedited procedure for epi-off (10 minutes, 9 mW/cm²) (288.46 ± 42.37 mm) (P , 0.05).

Mazotta et al[5] investigated half of patients who were treated with pulse light and the other half continued light. Irradiance was done with a 30 mW/cm² dosage of 7.2 J/cm² for 4 minutes. The demarcation line depth was shown in the pulsed light treatment to be meanwhile of 215µm (range: 190–235µm), with continuous light accelerated treatment revealing an average penetration of 160µm (range: 150–180µm).

Kymionis et al. [6] conducted an epi-off CXL research. The targeted irradiance of 9.0mW/cm² corresponding to the total surface dosage of 5.4 J/cm² was treated for 10 minutes. The mean depth of the demarcation line for corneal stroma of Group 2 was 288.46 ± 42.37 mm (238.5 to 353.5 mm range).

Kymionis et al. [4] carried out a 14-minute CXL rapid epi-off with 9mW/cm² UV-

An intensity of radiation equivalent to a total surface dosage of 7.5 J/cm². For Group 2, the mean depth of the corneal current line was 322.91 ± 48.28µm.

In a Lhuillier et al research, [7] 75 eyes of 58 individuals were subjected to epi-off CXL using the expedited protocol (10 min, 9 mW/cm² ultraviolet A, the overall surface dosage of 5.4 J/cm²). In the 1-month optical coherence tomography study, the mean stromal DL depth was 331.2 ± 62.7 µm.

Yam et al, [8] carried out a CXL epi-off on 43 eyes of 30 patients. If the corneal stroma is less than 400 µm, the swelling of the cornea has taken place every 2 minutes for 30 minutes with clean water drops. An irradiation of 3.0 mW/cm² anticipated for 30 minutes (surface dosage 5.4 J/cm²). The median CXL demarcation line depth at the central cornea for swellings and non- swelling groups was 297.5±60.8 µm (range: 180 to 360 µm), and 323.0±48.6 µm (range: 217 to 397 µm), respectively.

12 eyes accelerated epi-off CXL in a research by Ng Al et al [9]. They got 9 mW/cm², 10 minutes. The limit was 209.1 ±49.6µm.

Regarding the transepithelial method, we found DL average depth of 190 ±20 microns (P-value <0.001), close to Artola et al 2017, which analysed a total of 19 keratoconous eyes of 12 patients who were subject to accelerated transepithel CXL. Ultraviolet light for 2min and 40s was administered. The total irradiation energy was 7.2 J/cm² with a 45 mW/cm² UV power. The cornea was washed with a balanced saline solution after irradiation. The mean limit depth of the OCT-measured demarcation line ranged from 153 to 230 µm with a mean value of 205,19 ±205 µm).

The study at Abdel-Radi, M., et al. [10] has shown that Accelerated Epi-off CXL's mean corneal DL depth was 219.9±58.4 and 127.2±7.8 µm, with significant difference in trans-epithelium CXL (P<0.05).

Liu et al. [11] gathered eligible studies from four electronic databases, CENTRAL, PubMed, OVID MEDLINE and others. Twenty-four comparative studies on cross-linking were included in comparison with conventional cross-linking. The normal cross-linking demarcation line was much deeper than that of transepithel cross-linking, the median difference being -133.49. DDL. [3] [3]

Our research compared three most prevalent corneal crosslinking techniques with a non-invasive measurement instrument (AS-OCT)

The key constraint was the tiny number of situations that could be compensated for by the substantial variation in demarcation line depth.

One of CXL's most often discussed issues is to establish new and accurate measures for measuring the efficiency of the intervention. The interpretation of the demarcation line depth is now regarded an indirect assessment of the CXL penetration of the stroma,

therefore an indirect sign of efficacy therapy. The emphasis of this dispute was on the association between treatment efficiency and the depth of the corneal current demarcation line. The actual change of the corneal collagen after the photochemical CXL reaction still need to be understood.

The demarcation line depth difference between epi-off procedures and epi-on procedures is due to the existence of two factors: protecting the intact epithel and reducing the penetration of riboflavin stroma during a soaking phase. Thus, the corneal stromal demarcation line was much deeper in comparison with epi-on transepithelial procedures following standard epi-off procedures. This is why CXL iontophoresis provides better penetration into the stroma and hence higher effectiveness and deeper demarcation than the transepithelial epi-on method, which is more akin to the epi-off procedures. The difference in profundity of the line of demarcation between the epi-off (standard CXL and accelerated epi-off) and the epi-on procedures might be related to the protective effect of the later technique's undamaged corneal epithelium, which decreases the penetration of UVA. [2]

The doctor can help to better understand the efficacy of CXL therapy in horns of varying thicknesses in selecting the appropriate CXL procedure for each instance. The literature shows a reduced stabilising impact in a shallower cross-linking procedure. To that end, monitoring how deeply the therapy gets into each cornea may help to stabilise keratoconus and iatrogenic keratectases as an indication of efficacy. [2]

5. Conclusion

After study of several corneal crosslinking protocols, we have shown that stromal demarcation lines have shown more depth in standard epidips than accelerated epi-offs, whose depth is higher than transepithel crosslink, which means that standard epidips, accelerated epidips and transepithelis mean more penetration of therapy.

References

- [1] A. Mesen, B. Bozkurt, U. Kamis, and S. Okudan, "Correlation of demarcation line depth with medium-term efficacy of different corneal collagen cross-linking protocols in keratoconus," *Cornea*, vol. 37, pp. 1511–1516, 2018.
- [2] L. Spadea and R. Mencucci, "Transepithelial corneal collagen cross-linking in ultrathin keratoconic corneas," *Clin. Ophthalmol. (Auckland, NZ)*, vol. 6, pp. 1785, 2012.
- [3] Y. Salah, K. Omar, A. Sherif, and S. Azzam, "Study of demarcation line depth in transepithelial versus epithelium-off accelerated cross-linking (AXL) in keratoconus," *J. Ophthalmol.*, vol. 2019, pp. 656-661, 2019.
- [4] G. D. Kymionis et al., "Correlation of the corneal collagen cross-linking demarcation line using confocal microscopy and anterior segment optical

- coherence tomography in keratoconic patients,” *Am. J. Ophthalmol.*, vol. 157, pp. 110–115, 2014.
- [5] C. Mazzotta, C. Traversi, A. L. Paradiso, M. E. Latronico, and M. Rechichi, “Pulsed light accelerated crosslinking versus continuous light accelerated crosslinking: one-year results,” *J. Ophthalmol.*, vol. 2014, pp. 431–442, 2014.
- [6] G. D. Kymionis, K. I. Tsoulnaras, D. A. Liakopoulos, C. A. Skatharoudi, M. A. Grentzelos, and N. G. Tsakalis, “Corneal stromal demarcation line depth following standard and a modified high intensity corneal cross-linking protocol,” *J. Refract. Surg.*, vol. 32, pp. 218–222, 2016.
- [7] L. Lhuillier et al., “Visibility and depth of the stromal demarcation line after corneal collagen cross-linking using anterior segment optical coherence tomography: comparison between isoosmolar and hypoosmolar riboflavin,” *Cornea*, vol. 37, pp. 567–573, 2018.
- [8] J. C. S. Yam, C. W. N. Chan, and A. C. K. Cheng, “Corneal collagen cross-linking demarcation line depth assessed by Visante OCT after CXL for keratoconus and corneal ectasia,” *J. Refract. Surg.*, vol. 28, pp. 475–481, 2012.
- [9] A. L. K. Ng, T. C. Y. Chan, and A. C. K. Cheng, “Conventional versus accelerated corneal collagen cross-linking in the treatment of keratoconus,” *Clin. Experiment. Ophthalmol.*, vol. 44, pp. 8–14, 2016.
- [10] M. Abdel-Radi, Z. Eldaly, H. Abdelmotaal, R. Abdelrahman, M. Sayed, and K. Soliman, “Correlation between corneal demarcation line depth in epithelium-off and trans-epithelium accelerated corneal cross linking and keratoconus progression,” *Int. J. Ophthalmol.*, vol. 13, p. 907, 2020.
- [11] Y.-C. Liu, N. C. Lwin, N. S. W. Chan, and J. S. Mehta, “Use of anterior segment optical coherence tomography to predict corneal graft rejection in small animal models,” *Invest. Ophthalmol. Vis. Sci.*, vol. 55, pp. 6736–6741, 2014.