

## Research article

# Evaluation of corneal demarcation line and associated parameters in patients with keratoconus following transepithelial, epithelial scoring and epithelium-off accelerated corneal collagen crosslinking

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*The Journal of the College of Ophthalmologists of Sri Lanka* 2022; 28: 71-75

## Abstract

**Introduction:** Keratoconus is a progressive corneal ectatic disorder with thinning, protrusion and poor vision. Corneal collagen crosslinking (CXL) is a minimally invasive procedure used to prevent progression. CXL is performed either after epithelial debridement (Epi-Off) or with the epithelium in-situ (transepithelially).

**Objective:** To evaluate corneal demarcation line and associated parameters in patients with keratoconus following accelerated CXL using the described methods.

**Materials and methods:** Retrospective data of twelve consecutive eyes (n=12) which underwent CXL using the method described by the authors was compared with randomized data of sixteen eyes (n=11) which underwent transepithelial CXL and eighteen eyes (n=14) which underwent Epi-Off CXL. An accelerated CXL protocol was used (UVA 9mW/cm<sup>2</sup> for 10 min) in all patients after instillation of 0.1% riboflavin solution for 30 minutes. The surgeon made linear full thickness epithelial abrasions in the modified technique. Visual acuity, keratometry, pachymetry were evaluated preoperatively and at one month postop. Specular microscopy and AS-OCT was performed at one month postop.

**Results:** Preoperative Kmax, Kmean and cylinder power were similar in all groups. However, patients who underwent the modified technique had poorer UCDDVA and BCDVA at baseline (MRSEQ-4.41) and average pachymetry of 450  $\mu$ m. Postoperatively, patients who underwent the modified technique had mild stromal haze, a well visible stromal demarcation line on AS-OCT and a higher average endothelial cell count on specular microscopy. Keratometry and pachymetry values were stable in all groups.

**Conclusion:** Preliminary data indicate that the method described by the authors can be used in thinner corneas to improve efficacy of CXL compared to the transepithelial approach.

**Key words:** corneal ectasia, keratoconus, transepithelial collagen crosslinking, epithelial scoring technique

## Introduction

Keratoconus is an ectatic disorder where the central or paracentral cornea undergoes progressive protrusion and thinning. This causes the cornea to assume a cone shaped configuration<sup>1</sup>. It is a common disorder with a high prevalence seen in South Asia. Surveys show it to be up to 7.5 times more commoner among immigrant South Asians than Caucasians in mixed western populations<sup>2</sup>.

The characteristic non-inflammatory nature of thinning is possibly due to alteration in the regular orthogonal orientation of corneal stromal collagen fibers resulting in biochemical instability of the tissue<sup>3</sup>. In fact, studies using femtosecond laser slicing of the cornea into thin sections and x-ray diffraction with 3D modelling of stromal collagen have found the posterior two thirds of the stroma to contain collagen lying predominantly in the horizontal and vertical meridians, which may resist deformation of the cornea by the pull of the horizontal and vertical rectus muscles and the anterior one third to contain more isotropic collagen which may resist intraocular pressure and maintains the corneal curvature<sup>4</sup>. This modelling may explain why fragmentation and breaks in the Bowmans layer and thinning of the overlying corneal epithelium is seen clinically as well as on topography and pachymetry in patients with keratoconus.

However, researchers have also found differential involvement of the layers of the cornea in the ectatic process. The anterior corneal astigmatism has been shown to be higher than the posterior corneal astigmatism in late stages of keratoconus and the posterior cornea has shown to be affected much early

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in the disease process<sup>5</sup>. This lack of understanding of the exact pathophysiology has eluded a definitive treatment for progressive keratoconus.

Corneal collagen crosslinking (CXL) is currently regarded as the treatment of choice for prevention of progression in keratoconus as well as in other corneal ectatic diseases, especially in younger patients whom have a higher rate of progression. The original protocol described by researchers of the University of Dresden involves removal of the corneal epithelium under topical anesthesia, instilling riboflavin 0.1% in 20% dextran solution to the corneal surface for 30 minutes and applying UVA light of 365-370 nm and irradiance of 3 mW/cm<sup>2</sup> for 30 minutes at a 1 cm distance from the eye. Riboflavin is usually reinstalled every 5 minutes during the UV irradiation process as well. Currently, most surgeons use an accelerated crosslinking protocol where the total energy delivered is kept constant by using a higher fluence of UVA for a lower duration according to the Bunson-Roscoe law of reciprocity. This reduces the time duration of the procedure whilst maintaining comparable efficacy<sup>6</sup>.

The mechanism of stromal strengthening is by riboflavin acting as the photosensitizing agent and UV light as the energy source to release singlet oxygen free radicals from reacting atmospheric oxygen molecules to cause photo-polymerization of stromal collagen and forming strong chemical bonds with adjacent fibrils. This reduces the lytic effect of corneal collagenase and increases corneal resistance to deformation. Riboflavin induced crosslinking therefore acts as a hastening event in the natural age induced crosslinking of stromal collagen that precedes the cessation of progression in keratoconic patients during their middle ages.

To mitigate the toxic effects of UV light on the endothelium, the corneal stroma should be of adequate thickness. The exact corneal thickness which obviates endothelial damage during crosslinking has not been identified; however a residual corneal thickness of 400 µm after epithelial debridement is accepted as the safe limit based on animal studies. This cutoff value was used to select patients in the original Dresden protocol.

The limitation, however, is that a significant proportion of patients with keratoconus have corneas thinner than 400 µm. This proportion seems to be high among our cohort of Sri Lankan patients. To circumvent this limitation, transepithelial crosslinking was described. However studies have shown this to be 70-80% less effective than the epithelium-off (Epi-off) approach. The presence of the corneal epithelium in situ is a physical

barrier for both riboflavin and UV-A light penetration to the site of pathology within the stroma<sup>7</sup>.

Over the years, several authors have described methods to improve the efficacy of transepithelial collagen crosslinking. These include using a hypotonic riboflavin solution to iatrogenically swell the cornea during the procedure, using an overlying contact lens or a SMILE lenticule as a protective barrier against UV light induced endothelial damage after epithelial debridement in thinner corneas and customized epithelial debridement techniques among others. Pachymetry guided epithelial debridement techniques which have been described, debride epithelium over areas of the cornea thicker than 450 microns and leaves an area of epithelium overlying the thinnest point of the cornea. This "epithelial island" crosslinking technique has demonstrated adequate safety and efficacy in clinical trials<sup>8</sup>. However, all of the above are either technically challenging, require sophisticated equipment or are not cost effective in low to middle income countries with limited resources. Therefore the authors describe a modified transepithelial crosslinking technique to improve the efficacy of CXL in patients with thinner corneas not suitable for epi-off CXL.

## Objectives

- To compare outcomes of the described epithelial scoring method vs accelerated Epi-off CXL vs accelerated transepithelial CXL to demonstrate whether the epithelial scoring technique improves efficacy of accelerated CXL.
- To describe the preoperative and postoperative corneal biometrics in patients who underwent accelerated CXL using the different protocols.
- To describe the presence of demarcation lines and stromal haze on postoperative AS-OCT images after CXL to be used as a surrogate for efficacy of CXL.

## Methods

We designed a descriptive comparison study of 46 eyes of 37 patients with keratoconus who underwent accelerated CXL using the 3 different protocols during January to September 2022. CXL was performed by a single surgeon at a single center. Informed written consent was obtained from the participants. The protocols and study was designed in compliance with the WMA declaration of Helsinki. Patients who did not consent to participate in the study were excluded.

Participants were recruited under 3 categories:

1. Fourteen patients (18 eyes) undergoing accelerated Epi-Off CXL were recruited using simple random sampling.
2. Eleven patients (16 eyes) undergoing transepithelial CXL were recruited using simple random sampling.
3. Twelve patients (12 eyes) undergoing accelerated CXL with the author described method were recruited consecutively using non-random convenient sampling.

Preoperative uncorrected and best-corrected distance visual acuity, Scheimflug imaging based corneal topography and tomography and OCT based corneal pachymetry measurements of the study participants were recorded. Postoperative data that were collected included postop uncorrected and best-corrected distance visual acuity, repeat Scheimflug imaging based corneal topography and tomography, postop OCT based pachymetry, anterior segment OCT images of the cornea and specular microscopy of the corneal endothelium.

### Surgical technique

All patients underwent accelerated CXL under topical anesthesia. Sterile preparation and draping of the surgical field was done in all patients. Epi-Off CXL eyes underwent alcohol assisted epithelial debridement prior to riboflavin instillation. Transepithelial CXL eyes proceeded with riboflavin instillation without epithelial debridement. In eyes undergoing the modified technique, the surgeon made linear full-thickness patterned scratches on the epithelial surface. All eyes thereafter were treated with 0.1% riboflavin in 20% dextran solution for 30 minutes followed by 10 minutes of UVA 370nm irradiation at 9mW/cm<sup>2</sup>. Prophylactic antibiotic drops, steroid drops and a bandage contact lens was placed on all eyes at the end of the procedure.

### Results

The average age of the study participants undergoing the epi-off, transepithelial and the modified technique were 18.5, 22.4, 23.8 years respectively with each group have a mean preoperative uncorrected distance visual acuity of 0.6, 0.8, and 1.0.

Table 1 shows the preoperative corneal metrics.

**Table 1. Preoperative corneal metrics**

|                                | <b>Epithelial Scoring</b> | <b>Transepithelial</b> | <b>Epithelial Off</b> | <b>95%CI</b>         |
|--------------------------------|---------------------------|------------------------|-----------------------|----------------------|
| Preop Mean Kmin                | 48.55                     | 47.48                  | 47.12                 | 47.369-51.651        |
| Preop Mean Kmax                | 52.61                     | 51.06                  | 50.89                 | 49.165-53.874        |
| Preop Mean K average           | 50.5                      | 49.17                  | 48.9                  | 47.395 – 51.651      |
| Preop MRSEQ                    | minus 4.41                | minus 3.55             | minus 3.76            | 0.610-5.749 (p<0.05) |
| Mean Pachymetry thinnest point | 450.7 µm                  | 435.8 µm               | 472.3 µm              | 407.3-498.5 (p<0.05) |
| Mean epi thickness             | 40 µm                     | 40 µm                  | 42.5 µm               |                      |

Table 2 shows the postoperative corneal metrics

**Table 2. postoperative corneal metrics**

|                                | Epithelial Scoring | Transepithelial   | Epithelial Off      | 95%CI       |
|--------------------------------|--------------------|-------------------|---------------------|-------------|
| Postop Mean Kmin               | 49.4               | 48.15             | 47.91               |             |
| Postop Mean Kmax               | 55.2               | 52.87             | 52.74               |             |
| Postop Mean K average          | 52.3               | 50.51             | 50.23               |             |
| Postop average astigmatism     | minus 5.3          | minus 4.23        | minus 4.8           | 3.44-6.10   |
| Mean Pachymetry thinnest point | 470 $\mu\text{m}$  | 423 $\mu\text{m}$ | 458.3 $\mu\text{m}$ | 389 -511    |
| Mean epi thickness             | 43 $\mu\text{m}$   | 40 $\mu\text{m}$  | 44.8 $\mu\text{m}$  | 0.347-6.232 |

Evaluation of post CXL stromal demarcation line showed a faintly visible line in the transepithelial group whilst the other two groups showed a well visible demarcation line. All 3 groups had a post-operative central endothelial density of at least 2600 cells/ $\text{mm}^2$ .

## Discussion

The results above show that the epithelial scoring CXL method showed stable postoperative corneal biometrics with good efficacy and acceptable safety. However, this study had several limitations. The presence and strength of the stromal demarcation line was taken as a surrogate for efficacy. For true efficacy, documentation of lack of long term keratoconus progression must be demonstrated. The limited duration of follow-up in this study precludes this. Also patients who underwent epithelial scoring CXL were selected to the study by non-randomized sampling due to inherent nature of the design of the study. Appropriate probability sampling of a larger sample size will be necessary for more generalization of the study results. Also, the use of postoperative specular microscopy parameters as a surrogate for safety of the modified procedure is not absolute evidence of the lack of any safety concerns. Long term followup is necessary for further safety evaluation of the modified technique.

## Conclusion

The described modified CXL technique is an acceptable, less technically demanding and cost effective method to improve efficacy of transepithelial collagen crosslinking in patients with progressive keratoconus. This method shows efficacy similar to epi-Off CXL and therefore is an option for patients with thin corneas less than 400  $\mu\text{m}$  not amenable to epi-Off CXL.

## Author declaration

Authors declare that there is no conflict of interest.

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