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# Corneal Stromal Demarcation Line Depth after Corneal Collagen Cross-Linking Using Customized vs. Conventional Corneal Epithelial Debridement

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## Abstract

**Aim of the Study:** Compare the corneal demarcation line (DL) depth after corneal collagen cross-linking (CXL) with subtotal versus customized corneal epithelium debridement using anterior segment optical coherence tomography (AS-OCT).

**Design:** Prospective case-control study.

**Materials and Methods:** The study enrolled 18 patients with bilateral progressive keratoconus. Both eyes treated by CXL using 3 mW/cm2 / 30 minutes setting. One eye with subtotal epithelial debridement (about 9 mm diameter) and the contralateral eye with customized debridement (approximately 1 mm single horizontal central scratch). One month postoperatively, patients had AS-OCT imaging to detect and measure the depth of DL.

**Results:** Patients' age mean was 25.17 years +/- 4.81 SD. Epithelial healing completed within 3-7 days in conventional treatment group and demarcation line was evident in 16 eyes (89%) with a mean depth of 290.31 µm while in customized debridement group, the epithelial healing lasted less than 24 hours and DL was detectable in 10 patients (55.5 %) with a mean DL depth of 221 µm with a statistically significant difference ($p<0.05$). Subjective postoperative pain graded as "moderate to severe" in about 77% of eyes underwent 9 mm epi-off CXL compared with 55% of those with customized debridement group.

**Conclusions:** Although the DL if found in majority cases, but the shallow location in customized corneal debridement cases questioned the efficacy of this technique despite the quick re-epithelialization and less postoperative pain that accompanied it.

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## Introduction

Keratoconus is a bilateral, usually asymmetrical noninflammatory progressive ectatic corneal disorder that can cause a significant visual morbidly. It affect the central and paracentral cornea and the cornea progressively becomes conical in shape and it typically present in puberty and usually associated with progressive corneal thinning and irregular myopic astigmatism that may mandate transplantation [1].

Estimated incidence of keratoconus ranged from 1.3 to 22.3 cases/100000 and prevalence from 0.4 to 86 cases/100000 [2,3], this wide range of incidence is thought to be governed by a variety of genetic and environmental factors [4-6].

Keratoconus is a common eye problem in Middle East area, including the current study locality where atopy and vernal keratoconjunctivitis is endemic [7-10].

Since the introduction of collagen cross linking using ultraviolet radiation type A (UV-A) and riboflavin as a treatment option to limit the progressive keratoconus by Spoerl and Seiler at the University of Dresden [11], it gained a worldwide popularity as a novel conservative strategy to halt or decrease the progression of various corneal ectasia including keratoconus, pellucid marginal degeneration, post-LASIK ectasia and keratoglobus [12-14]. This technique reported to flatten the cornea stabilize the progression of irregular astigmatism [15].

With the standard protocol suggested (Dersden protocol) that involve debridement of the central 9 mm of the corneal epithelium and application of UV-A 3mW/cm$^2$ for 30 minutes (5.4 J/cm$^2$ dose) [15,16]. UVA excite to riboflavin to liberate a singlet oxygen molecule that causes the corneal collagen fibers cross linking through polymer chains connection to each other [17-19] keratocytes apoptosis reaches to a depth of about 300 µm leaving the posterior stroma intact [20] causing increased density of extracellular matrix and change in refractive index between the anterior treated stroma and posterior stroma that is visible within one month by slit lamp examination and called " Demarcation Line" and line is thought to be a clinical sign of the depth and effectiveness of CXL [21,22].

With the advances in anterior segment optical coherence tomography (AS-OCT) it become feasible to demonstrate the presence of DL and measure the depth with the built-in calipers. The corneal epithelium physiologically acts to protect the underlying stroma due to the presence of tight junctions between its cells that preclude the intrusion of microorganisms and at the same time retard the penetration of riboflavin molecules [23], so during this protocol, the de-epithelialized corneal stroma in immediate postoperative period is subjected to the attendant risk of bacterial keratitis, delayed epithelialization, persistent haze, sterile corneal infiltrate [24], at the same time, the exposed corneal nerve endings causes variable about of pain that may be severe enough to need systemic administration of potent analgesia.

The central corneal stroma thickness is pathologically thinned in advanced keratoconus, thus in case of subtotal epithelial debridement there's risk of UVA-induced corneal endothelial cytotoxicity specially if the residual stromal thickness is below 400 μm.

Other protocols have been suggested to replace the conventional subtotal epi-off CXL to decrease the risk of postoperative bacterial keratitis and to provide a rapid visual rehabilitation and less postoperative pain. Suggested alternative protocols included performing CXL with intact epithelium (epi-on CXL) with or without iontophoresis that electrically direct the negatively charged riboflavin molecule into the corneal stroma and in this protocol the corneal epithelium is not breached and the need for debridement is abolished with sufficient penetration of riboflavin molecule into the corneal stroma [14,25].

But previous studies questioned the efficacy of epi-on CXL and revealed the shallow on non-evident corneal demarcation line [26-30]. The corneal epithelium tight junctions thought to retard the absorption of riboflavin by the corneal stroma and thus decrease the efficacy of epi-on procedure, in addition, adequate oxygen is vital for effective cross linking is decreased as the epithelium acts as a barrier [31], also the corneal epithelium metabolic activity far exceed the stroma so the oxygen needed is consumed by the intact epithelium in epi-on procedures which is a crucial factor for successful crosslinking [32].

We suggested performing corneal cross linking with a customized limited epithelial debridement much less than the classical subtotal 9mm diameter to decrease the immediate postoperative pain and time needed for complete epithelial healing , hence decrease the risk of postoperative bacterial keratitis and at the same time gain the advantage of opening a path for the riboflavin to pass unimpeded by the corneal epithelium tight junctions and thus decrease the time for visual rehabilitation without compromise the effectiveness of the corneal stiffening.

## Aim of Study

Compare the clarity and depth of corneal stromal demarcation line in subtotal versus limited epithelial corneal debridement before CXL using anterior segment optical coherence tomography.

## Patients and Methods

This is randomized prospective study conducted in Ophthalmology Center of Basra Teaching Hospital, South of Iraq. Thorough explanation of the research was given to all patients and they signed an informed following the ethical committee of the College of Medicine, University of Basra.

The data collection lasted from February 2018 to October 2019. This study enrolled 18 patients with documented bilateral progressive keratoconus who had grade I to III according to Amsler-Krumeich classification [33,34]. Progression criteria was one or more of the following:

Increased keratometry reading by 1D or more

- Refractive error changes (> 0.5 D sphere or > 1.0 D cylinder)
- Decreasing best corrected visual acuity (BCVA) by two lines or more

Exclusion criteria:

- Keratometry reading > 60
- Central Corneal thickness <400 μm
- Corneal epithelial healing disorders
- Previous herpes keratitis
- Corneal scar
- Corneal melting disorders
- Pregnancy

All patients underwent a thorough ophthalmological examination including BCVA, slit lamp examination, tonometry and baseline corneal topography using Scheimpflug camera device (Sirius, CSO).

Patients underwent successive CXL of both eyes in same session. under sterile aseptic technique. After adequate topical anesthesia with 0.5% Proparacaine drop 2 drops every 5 minutes for 15 minutes, one eye underwent subtotal debridement of central 9 mm of the corneal epithelium and the contralateral eye underwent a limited rebridement of a narrow horizontal line of central corneal that does not exceed 4x1 mm dimensions using a small corneal scraper then the corneal stroma is soaked with riboflavin (Ricrolin, Sooft) 0.1% solution in 20% Dextrose one drop every 3 minutes for 30 minutes. After disinfection and draping, lid speculum is inserted and the eye is irradiated using UVA 370 nm (CBM Vega X-linker, CSO) with setting of 3.0 mW/cm$^2$ energy and 8 mm beam diameter for 30 minutes during this period the patient is asked to fixate on the flashing target light, during which time continue riboflavin drop every 5 minutes and topical anesthesia when needed.

Postoperatively, bandage contact lens fitted after adequate rinsing of riboflavin with Sodium Chloride 0.9% saline and the patient prescribed Tobramycin drop 0.3% solution (Tobrex, Alcon) and oral analgesics on need and followed closely waiting for complete corneal healing when the bandage contact lens is removed topical steroids and antibiotic mixture (Tobramycin 3 mg and Dexamethasone 1mg suspension) (Tobradex, Alcon) drop prescribed 4 times daily for 2 weeks and tapered over 1 month. Patients asked to describe the pain out of 3 descriptions (mild, moderate and severe) and the time needed for complete re- epithelialization is recorded in days.

Anterior segment OCT imaging using Nidek RS-3000 platform is performed at the end of first postoperative month to evaluate the clarity and depth of corneal stromal demarcation line and the depth measured by a built-in caliper. The three researchers evaluated the clarity and depth of the DL and questionable clarity is excluded from the study statistics.

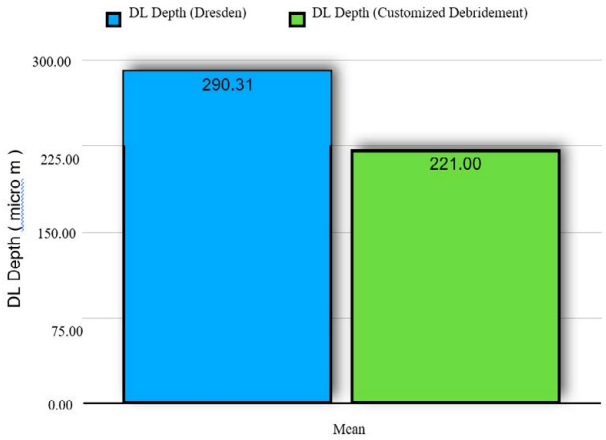
## Statistical Analysis

Analysis performed using SPSS software (SPSS, Inc.) version 24 and

Numbers software (Machintosh). We used means, standard deviation, range and Mann-Whitney *U* tests. P value of less than 0.05 considered statistically significant.

## Results

Patients' age mean was 25.17 years +/- 4.81 SD. The corneal epithelium healed within 3-7 days in 9 mm debridement CXL, on the other hand, all eyes with customized debridement technique healed within 24 hours. Subjective postoperative pain graded as "moderate to severe" and needed potent analgesia in about 77% of eyes underwent 9 mm debridement CXL compared with 55% of those with customized treatment. In conventional treatment group, corneal DL was evident in 16 eyes (89%) with a mean depth measurement of 290.31 µm while in customized debridement group, corneal DL was detectable in 10 patients (55.5%) with a mean DL depth measurement of 221.00 µm which was statistically significant different ($p<0.05$). None of the enrolled patients developed a significant postoperative complication like bacterial keratitis or persistent epithelial defect.




## Discussion

Efficacy of CXL is well documented in vitro by Wollensak G, et al. (2003) who demonstrated increased rigidity of treated human cornea by 4.5 times [35], but this experiment is not suitable for clinical testing of the efficacy of CXL as it used a stripped corneal tissue, so clinical indications of effectiveness are suggested including confocal microscopy and density and depth of DL using AS-OCT.

In this study, out of enrolled 18 patients underwent classical CXL by Dresden protocol with 9 mm de-epithelialization, 2 eyes only had indiscriminate DL with faint anterior stromal hyper reflectivity (about 11% of cases), on the other hand, 5 eyes with customized epi-off cases did not show a clear DL (about 28%) of cases.

The statistically shallow DL in customized debridement CXL group reflects decreased volume of corneal tissue treated and indicated relatively less efficacy than those eyes treated with conventional protocol. Corneas with customized debridement technique showed less DL depth and previous reports demonstrated that a shallow DL give a clue about the effectiveness of CXL and its stabilizing effect [36,37].

Shallow demarcation line in this study results may reflect the decreased corneal tissue volume subjected to the direct exposure to UV-A impeded by the intact epithelium due to the limited area of debridement and this reflect the relative decrease in efficacy of customized debridement CXL. This result coincides with other previous studies assessed the efficacy of different CXL protocols [26-30].

Kaya V, et al. (2011) investigated customized debridement CXL in which they preserved the epithelium over critically thin cornea as a trial to decrease the corneal endothelial cells damage and showed limited area of keratocytes apoptosis studied by confocal microscopy with absence of demarcation line beneath the area of intact epithelium [29].

This result demonstrates that the conventional CXL is still the most effective strategy for sufficient corneal stiffening to halt KC progression and customized epithelial debridement is still to be revised. The study reliability needs to be strengthened in view of the small sample size.

## Conclusion

Customized debridement group showed rapid healing with less pain but had less mean DL depth and more eyes had no evidence of DL development compared to classical 9 mm epithelial debridement group.

## Disclosure

We have no financial interest to disclose.

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