• Clinical Research •

Correlation between corneal demarcation line depth in epithelium-off and trans-epithelium accelerated corneal cross linking and keratoconus progression

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Abstract

● **AIM:** To compare corneal demarcation line (DL) depth in both accelerated epithelium-off and trans-epithelium cross linking (CXL) using anterior segment optical coherence tomography (AS-OCT) and its relation to maximum keratometry (Kmax) progression in both techniques.

● **METHODS:** A prospective comparative interventional study where patients with mild to moderate keratoconus (KC) were classified into two groups: accelerated epithelium-off and trans-epithelium CXL based on corneal pachymetry. Assessment of corneal DL depth was carried out after 3mo by AS-OCT. Kmax readings were evaluated after one year follow up using the Scheimpflug imaging system.

● **RESULTS:** Study included 74 eyes of 44 patients. Group A underwent epithelium-off CXL (41 eyes), while Group B underwent trans-epithelium CXL (33 eyes). At 3mo follow up, mean corneal DL depth in Group A was 219.9±58.4 μm while in Group B was 127.2±7.8 μm (P<0.05). The mean Kmax changed from 51.9±3.9 to 51.3±4.2 diopters in Group A and from 53.1±4.1 to 53.6±5 diopters in Group B with insignificant difference in Kmax changes in either group (P>0.05). In addition, no significant change in corneal pachymetry was found in both groups (mean change at 1y: 6.4±4.7 and -10.1±2.3 μm in Groups A and B respectively).

● **CONCLUSION:** Despite a significantly deeper corneal DL depth created by accelerated epithelium-off CXL technique compared to accelerated trans-epithelium CXL, there is no significant impact on keratoconus progression.

● **KEYWORDS:** anterior segment optical coherence tomography; cross linking; demarcation line; keratoconus

INTRODUCTION

Keratoconus is a progressive corneal degenerative disease resulting in corneal thinning and protrusion. Visual acuity significantly decreases due to astigmatism that becomes irregular with progression of the disease[1]. Management of keratoconus depends on a variety of factors including visual acuity, the degree of corneal thinning and steepening[2]. Corneal collagen cross linking (CXL) is now considered as the treatment of choice in mild to moderate cases of keratoconus and is proven to halt the disease progression[3-4]. The implantation of intra-stromal corneal ring segments has been indicated for cases with moderate keratoconus, while advanced cases of keratoconus are candidates for deep anterior lamellar or penetrating keratoplasty (DALK or PKP)[5-6].

CXL idea was based on the fact that a photosensitizer substance like riboflavin (vitamin B2) can interact with ultraviolet irradiation (ultraviolet-A) to strengthen the corneal tissue inter and intrafibrillar collagen bonds thus preventing further thinning, corneal protrusion and reduces corneal irregular astigmatism[7]. Epithelial debridement enhances riboflavin corneal penetration that allows absorption of wide range of light spectrum wave lengths including ultraviolet A[8]. The idea of trans-epithelial delivery of riboflavin into the corneal tissue was hindered by the fact that riboflavin can't penetrate intact corneal epithelium. The addition of certain molecules such trometamol allows penetration of riboflavin into the corneal stroma that markedly reduces the possible complications of removing of the corneal epithelium (Epi-off technique) such as persistent epithelial defects, scarring and serious infectious keratitis. Another advantage of trans-

epithelial CXL that it reduces the cytotoxic effects of ultraviolet irradiation on corneal endothelium and intraocular structures especially in thin corneas less than 400 μm[9-10].

CXL induces stromal collagen fiber shrinkage. Ultraviolet A exposure enhances covalent bond formation between collagen fibers especially in the anterior stroma where 65% of ultraviolet irradiation is absorbed within first 250 μm thus a hyperrefelctive transitional area can be detected between the anterior cross linked and the posterior untreated corneal stromal tissue referred to as a demarcation line (DL) that is usually evident 1-6mo after CXL procedure[11-12].

A comprehensive slit lamp examination could detect the DL, however anterior segment ocular coherence tomography (AS-OCT) is a more sensitive tool to assess the extent and depth of a stromal DL that is deeper centrally than peripherally due to the natural corneal curvature[13-14].

Most of the previous studies paid attention only to compare demarcation line depth measurements in different CXL protocols and the question that is debatable "Does a deeper demarcation line represent a more efficient cross linking?"[15].

In our study we investigated the difference in corneal DL depth and percentage in accelerated Epi-off and trans-epithelial CXL techniques and whether such difference was reflected on maximum keratometry (Kmax) in both techniques as an indicator for keratoconus progression.

SUBJECTS AND METHODS

Ethical Approval All patients were fully informed about the study concerning the aim, possible benefits and complications of CXL procedure, and signed a written informed consent. The study was conducted under the tenets of the Declaration of Helsinki and after approval of the Ethical Committee of Faculty of Medicine, Assiut University, Egypt. Clinical Trial Registry: ClinicalTrials.gov Identifier: NCT03879421.

Study Design This study was designed as a prospective comparative interventional study and included 74 eyes of 44 patients conducted at TIBA Eye Center, Assiut, Egypt.

Patient Selection Patients with progressive mild to moderate keratoconus (Kmax between 46 and 56 D), clear cornea and corneal pachymetry >370 μm were included. Progression was documented as an increase of Kmax more than 1 D over a year. Exclusion criteria were patients with advanced keratoconus (Kmax>56 D), corneal scarring, corneal pachymetry (thinnest location) <370 μm, post laser-assisted *in situ* keratomileusis (LASIK) ectasia and previous corneal surgeries *e.g.* intrastromal corneal ring segments.

Baseline Assessment All patients underwent a comprehensive ocular examination including uncorrected and best-corrected distant visual acuity (BCVA), intraocular pressure (IOP) measurement, slit lamp and fundus examination. The Scheimpflug imaging (Oculus Pentacam, Oculus Inc., USA) was considered as the golden tool for diagnosis of keratoconus based on the following parameters: corneal topography and astigmatism (axial/sagittal curvature maps), Kmax value, corneal thinnest location (pachymetric map), corneal anterior & posterior elevation maps and Belin/Ambrósio enhanced ectasia display.

Patients in our study were classified into two groups according to corneal thickness values: Group A where patients had corneal thickness >400 μm (thinnest location) and Group B where patients had corneal thickness <400 μm (thinnest location). Patients in Group A were assigned to undergo accelerated Epi-off CXL procedure while patients in Group B were assigned to undergo accelerated trans-epithelial CXL procedure. All CXL procedures were done by the same experienced surgeon (Abdel-Radi M).

Technique of Accelerated Epi-off CXL After placing our patient in a supine position comfortably, a drop of topical anesthetic (Benoxinate Hydrochloride 0.4 mg) was instilled, and a lid speculum inserted. The corneal epithelium was removed manually using a hockey knife and then VibeX-Rapid (Riboflavin 0.1%, Hydroxypropyl methylcellulose HPMC, Avedro Inc., USA) was applied to cover the cornea completely every 2min for 10min. The patient's cornea was exposed to accelerated CXL with Ultraviolet A 10 mW/cm² for 9min and the instillation of riboflavin 0.1% was continued every 2min until the end of ultraviolet irradiation. A soft bandage contact lens was applied and removed after healing of the corneal epithelium 3-5d after surgery.

Technique of Accelerated Trans-epithelium CXL Each patient was lying comfortably in a supine position, a drop of local anesthetic (benoxinate hydrochloride 0.4 mg) was instilled and a lid speculum inserted. Paracel (riboflavin 0.25%, EDTA, Trometamol, phosphate buffered solution, Avedro Inc., USA) was applied to completely cover the corneal surface and instilled every 90s for a total of 4min and then VibeX xtra (Riboflavin 0.22%, phosphate buffered solution, Avedro Inc., USA) was instilled every 90s for a total of 6min. The patient's cornea was exposed to accelerated CXL with ultraviolet A 10 mW/cm² for 9min and was rinsed with a drop of balanced salt solution (BSS) every 2min during the procedure. A soft bandage contact lens was applied and removed after two day. All patients received topical moxifloxacin 0.5% (Vigamox®, Novartis Pharmaceutical Corp., USA) 4 times daily, topical tobramycin 0.3%/dexamethasone 0.1% (Tobradex®, Novartis Pharmaceutical Corp., USA) after epithelial healing 4 times daily for 2wk, in addition to ocular lubricants applied hourly in the first few days and gradually tapered over 3mo according to physician discern.

Patients in both groups were scheduled to have regular follow up visits and Pentacam was scheduled after one-year post-CXL

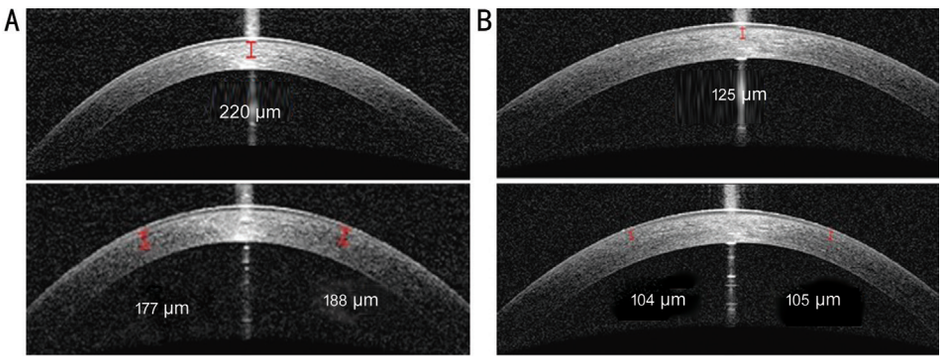

Figure 1 Two cases of progressive keratoconus in epithelium-off and trans-epithelium accelerated CXL groups respectively A: A 25 years old male with progressive keratoconus in his right eye. Corneal thickness was 475 μm and Kmax was 49.4 D. Epithelium-off accelerated CXL was performed. At 3mo follow up, DL depth was 220 μm (central), 188 μm (2 mm nasal) and 177 μm (2 mm temporal). At 1y follow up Kmax was 49.2 D. B: A 31 years old male with progressive keratoconus in his left eye. Corneal thickness was 384 μm and Kmax was 52.8 D. Trans-epithelium accelerated CXL was performed. At 3mo follow up, DL depth was 125 μm (central), 105 μm (2 mm nasal) and 104 μm (2 mm temporal). At 1y follow up Kmax was 53.0 D.

to assess keratoconus progression. Refractive, pachymetric and keratometric changes were recorded at baseline and at 1y post-CXL with special concern on post-CXL Kmax.

Anterior Segment OCT Technique and Measurements All patients underwent AS-OCT evaluation of the corneal DL 3mo post-CXL by Spectralis SD-OCT (Heidelberg GmbH, Germany). The Spectralis AS-OCT had an acquisition speed of 40 000 A-scans per second, with an axial resolution of 3.9 to 7 μm and a transverse resolution of 14 μm. A horizontal corneal line averaged to 9 frames was obtained and the demarcation line was identified in AS-OCT as a stromal line that separates a more reflective anterior corneal stroma from a less reflective posterior corneal stroma. The depth of DL was measured starting from a point on the epithelial side to a point on the line of separation between the more and the less reflective stroma using a manual software calibre tool provided by the manufacturer. Three points at the horizontal line scan were included (centre & 2 mm nasally and temporally) to detect the DL depth.

The percentage of the treated cornea (%) in relation to the preoperative corneal pachymetry was measured by dividing the volume of the treated cornea (DL depth) by the central corneal thickness and multiplying the result by 100 for each case. All AS-OCT measurements were taken by the same experienced ophthalmologist (Eldaly Z).

Statistical Analysis Quantitative data was presented in mean±standard deviation (SD). Statistical analysis was carried out by SPSS v. 20.0 (SPSS Inc., USA). Statistical significance was considered significant if $P<0.05$.

RESULTS

This study included 74 eyes of 44 patients where 30 patients had bilateral CXL and 14 patients had unilateral CXL. Of whom, 26 males (59%) and 18 females 41% with a mean age of 23.3±5.4 and 25.15±7.9y in Groups A and B respectively. Mean central corneal thickness of Groups A and B was 489.0±51.7 and 397.9±31.1 μm respectively. Demographic characteristics and baseline assessment are presented in Table 1.

Table 1 Demographic characteristics and baseline evaluation

Characteristics	Group A	Group B
No. of patients	25	19
Eyes	41	33
Age (y)		
Mean±SD	23.3±5.4	25.15±7.9
Range	18-36	19-39
Gender		
Male	16/25	10/19
Female	9/25	9/19
BCVA	0.23±0.05	0.15±0.11
Central corneal thickness (μm)		
Mean±SD	489.0±51.7	397.9±11.1
Range	403-523	371-392
Kmax (D)		
Mean±SD	51.9±3.9	53.4±4.2
Range	49.5-53.2	48.2-55.4

BCVA: Best corrected visual acuity; Kmax: Maximum keratometry; SD: Standard deviation.

Corneal Demarcation Line Depth At 3mo follow-up, the mean corneal DL depth of Epi-off CXL was 219.9±58.4 and 127.2±7.8 μm in trans-epithelium CXL with a significant difference ($P<0.05$; Table 2, Figure 1).

The percentage of the volume of the cornea treated was calculated by measuring DL depth in relation to the preoperative mean central corneal thickness in both groups. The mean percentage of DL depth showed a significant difference between the two groups (45.5%±13.1% and 32.2%±3.4% in Groups A and B respectively, $P<0.05$; Table 2).

The mean DL depth measured at 2 mm nasally from the center of the cornea in epi-off CXL study group was 192.7±52.9 μm which was not significant when compared to 2 mm temporally that was 197.2±53.2 μm (P>0.05). Furthermore, mean DL depth measured at 2 mm nasally from the center of the cornea in trans-epithelium CXL group was 111.2±7.1 μm which was insignificant when compared to 2 mm temporally that was 108.6±6.1 μm (P>0.05; Table 3, Figure 1).

Demarcation Line and Keratoconus Progression There was an insignificant change in Kmax among Epi-off CXL patients. Likewise, Kmax didn't show a significant change after 1y in trans-epithelium CXL (Table 4). Regarding pachymetry change at 1y follow up, there was also an insignificant change in corneal thickness. In Group A, corneal thickness increased slightly from 489.0±51.7 μm to 496.4±63.1 μm (mean change 6.4±4.7, P>0.05) while corneal thickness was slightly reduced in Group B from 397.9±31.1 to 386.6±45.9 μm (mean change -10.1±2.3, P>0.05).

Table 2 DL depth and percentage of DL depth at the center in Epi-off and trans-epithelium CXL groups

DL depth	Group A	Group B	P
Center (μm)			
Mean±SD	219.9±58.4	127.2±7.8	<0.05
Range	123-284	102-130	
Percentage (%)			
Mean±SD	45.5±13.1	32.2±3.4	<0.05
Range	24.1-65.7	26.1-38.9	

DL: Demarcation line.

Table 3 DL depth at the corneal center, 2 mm nasal and temporal in Epi-off and trans-epithelium CXL groups

DL depth	Group A	Group B
Center	219.9±58.4	127.2±7.8
2 mm nasal	192.7±52.9	111.2±7.1
2 mm temporal	197.2±53.2	108.6±6.1
Nasal-temporal difference (P)	>0.05	> 0.05

DL: Demarcation line.

Table 4 Comparison between Kmax at baseline and 1-year post-CXL

Kmax	Group A	Group B
Baseline	51.9±3.9	53.2±4.1
1y	51.3±4.3	53.7±5.0
Difference	-0.5±0.3	0.4±0.2
P	>0.05	>0.05

Kmax: Maximum keratometry reading.

DISCUSSION

CXL is considered the treatment of choice for mild to moderate progressive keratoconus with proven efficacy and safety in halting the disease progression[16]. Different CXL protocols are available including standard conventional Epi-off CXL, accelerated Epi-off CXL, accelerated trans-epithelium CXL and trans-epithelium CXL by iontophoresis[17].

Many studies considered the appearance of a demarcation line between the treated anterior corneal stroma and the posterior stroma that is best detected with AS-OCT as a sign of successful crosslinking procedure of the ectatic cornea[18].

In our study, we found that DL depth after 3mo following Epi-off accelerated CXL in 41 eyes was 219.8 μm which is comparable to Mazzotta *et al*[19] who detected DL one month after accelerated Epi-off CXL in 20 eyes with keratoconus at approximate depth of 215 μm. Moramarco *et al*[20] also detected DL at an average depth of 213 μm one month after accelerated Epi-off CXL in 60 patients with keratoconus.

Trans-epithelium CXL was our procedure of choice in patients when pachymetric values were less than 400 μm. The detection of demarcation after 3mo of trans-epithelium CXL was at a mean depth of 127.1 μm which slightly deeper when compared to Filippello *et al*[21] who detected DL two weeks after trans-epithelium CXL in 20 eyes with keratoconus at shallower level of an approximate depth of 100 μm. In our study, deeper DL depth compared to what was determined by Filippello *et al*[21] could be explained by the different time interval between the trans-epithelium CXL procedure and DL detection on anterior segment OCT.

We noticed that the corneal DL on AS-OCT was deeper in accelerated Epi-off CXL than trans-epithelium CXL as presented in Table 2. Bottos *et al*[22] suggested that intact epithelium in trans-epithelium CXL techniques diminish the ability of riboflavin and ultraviolet irradiation to penetrate the corneal stroma. Richoz *et al*[23] provided another explanation of deeper DL levels and consequent better cross linking effect of Epi-off CXL techniques based on the fact that the intact epithelium acts as a barrier to rapid oxygen diffusion into the corneal stroma and results in less effective corneal stromal cross linking.

In order to avoid bias that may result from the effect of including patients with corneal thickness less than 400 μm in trans-epithelium CXL and patients with corneal thickness more than 400 μm in Epi-off CXL on the mean DL depth, we calculated the percentage of DL depth compared to the preoperative corneal thickness as shown in Table 2. Spadea *et al*[24] also reported a higher percentage of demarcation line depth of 59.6% in accelerated Epi-off CXL compared to 34.4% in trans-epithelium CXL.

In the current study, we measured the DL depth not only in the center but also 2 mm nasally and 2 mm temporally along the AS-OCT horizontal line scan in both accelerated Epi-off and trans-epithelium CXL patients. We found that there was no significant difference between the depth of DL between the temporal and nasal sides in both groups, however the depth

of DL was slightly deeper in the center compared to 2 mm on nasal and temporal sides in both groups that could be attributed to the top hat beam profile of ultraviolet irradiation that doesn't respect the natural corneal curvature as proposed by Malta *et al*[14]. To our knowledge, this is the first study to compare the depth of DL on both nasal and temporal sides along the horizontal line scan in both Epi-off and trans-epithelium CXL patients.

Kmax values in our study showed a mean reduction of 0.5 D in accelerated Epi-off group that was not significant which agree with Sadoughi *et al*[25]. Other studies such as Ozgurhan *et al*[26] reported significant mean reduction of Kmax values among Epi-off CXL pediatric patients.

On the other hand, Kmax values among trans-epithelium CXL group showed an insignificant increase (+0.4 D) but these changes were not considered as progression of keratoconus. Out of 33 eyes, only 3 eyes (about 9%) showed progression of keratoconus evidenced by increase of Kmax value more than 1 D while the remaining 30 eyes showed stable Kmax values. Our results were comparable to Soeters *et al*[27] who reported stability of average Kmax values after one year follow up in 27 eyes out of 35 eyes who underwent trans-epithelium CXL.

Absolute value of corneal DL depth provided a fallacious indicator about the efficiency of both CXL techniques omitting the importance of corneal thickness. Alternatively, it is advisable to utilize percentage of DL which will reflect the true effect of CXL on the corneal layers. Despite disparity of percentage of DL between the 2 techniques (45% in epithelium off CXL and 32% in trans-epithelium CXL), there was no notable difference in keratoconus progression regarding Kmax change after 1-year follow up in both groups. This could be explained by the fact that major corneal bio-mechanics responsible for corneal strength are located in the anterior third of corneal stromal lamellae[28]. So, for a successful CXL procedure, anterior third of the cornea should be sufficiently cross linked to maintain corneal stability in keratoconus patients. We suggest that comparable success of both CXL techniques in halting keratoconus progression was attributed to the percentage of DL in both protocols that exceeded the anterior third of the corneal stroma, the most significant part of the cornea regarding corneal biomechanics. The recent techniques that could measure the corneal stromal stiffness and corneal biomechanical parameters should be correlated with the new automated software OCT DL detection studies to judge the efficacy of different CXL protocols[29].

In conclusion, both techniques of accelerated CXL proved to be effective in halting keratoconus progression over 1y. Though Epi-off technique provided deeper DL depth than trans-epithelium CXL, no significant changes in Kmax and corneal pachymetry were reported in both groups.

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