DISSCUSION

Corneal collagen crosslinking (CXL) has gained worldwide popularity over the past several years as an effective means of strengthening the cornea and thereby reducing, if not eliminating, the progression of ectatic disorders of the cornea.

From the patient's perspective, there is no question that CXL without removing the epithelium (epi-on) is more comfortable, safer, and generally preferable to CXL with the epithelium removed (epi-off). However, the reason for performing CXL is to stabilize keratoconus, and the reported clinical outcome and experimental results of epi-on treatments is still controversial.

The concept of epithelium-on CXL is not new. The main concerns with so-called transepithelial CXL (TCXL) are that standard riboflavin used in epi-off CXL cannot penetrate an intact epithelium and that the presence of the epithelium will block approximately 20% of the UV-A light from reaching the stroma. Both of these concerns are valid, but these assumptions may not be entirely correct. TCXL rely on the presence of chemical agents in the riboflavin formulation that loosen the epithelial barrier, thus allowing penetration of riboflavin into the stroma in presence of the epithelium.

One of the tools to assess the effectiveness of CXL is to detect the presence of demarcation line in the stroma of crosslinked corneas. AS-OCT produces a high-resolution image of the cornea in which tools can be selected to measure the distance between 2 points. Despite the subjective character of the measurement, this technique provides an accurate measurement of demarcation line depth.

In our study we discussed a comparison between epi on and epi off techniques as regarding the presence or absence of demarcation line and its depth, we discussed also early changes in pentacam results.

Our study was conducted on 40 eyes of 20 keratoconus patients with clear central cornea.Group I includes 20 eyes undergone epithelium off CXL with age ranged between 15 and 31 years with mean =23.10 ± 5.45, group IIA includes 10 eyes undergone transepithelial CXL using Medio Cross TE-Transepithelial solution and CCL-HE machine with age ranged between 13 and 29 years with mean =20.60 ± 6.07, and group IIB includes 10 eyes undergone trasepithelial CXL using Ricrolin TE and CCL-365 machine with age ranged between 20 and 35 years with mean =29.20 ± 7.16.

OCT results:

From our OCT results we found that demarcation line was detected in all epi-off CXL cases while in epi on cases the results were different; In group IIA the line was not detected except in 2 eyes which undergone epithelial scratches, while in group IIB the line was detected in all cases.There was no statistically different findings between group I and group IIB corneal stromal demarcation line depth at both nasal locations and temporal location at 2 mm. The line was significantly deeper in group I at central and temporal location at 1 mm. There was no statistically significant difference between group I and group IIB corneal stromal demarcation line depth to corneal thickness ratio at all locations.

Epithelium off results:

In our study, the average demarcation line depth detected after one month was about 250 μm (mean DLD at central location was 254 μm), which is comparable to the previously reported by Seiler and Hafezi, they biomicroscopically detected a thin stromal demarcation line at a depth of approximately 300 micron as early as 2 weeks after treatment. [124] Mazzotta and associates detected a stromal demarcation line using confocal microscopy at a depth 320 μm. [84] Muriel detected a stromal demarcation line with AS-OCT at a depth 313 μm at 1 month after surgery in 28 of 29 eyes. [143]

Caporossi et al performed confocal microscopy analyses in humans after CXL. Interestingly, they in vivo detected the effective depth of treatment by identifying distinct vertical and lateral transition areas at a depth of 270 to 330 μm . Here, the anterior (treated) stroma showed edema with only a few keratocyte nuclei and poor reflectivity, whereas the posterior (untreated) stroma showed regular keratocyte population and normal reflectivity.[90]

Epithelium on results:

- In group IIA (epi-on, Mediocross TE) the demarcation line was not detected and these findings were similar to other clinical and laboratory studies that have reported weaker or no effect of CXL using the epi-on method. [104] Hayes and associates reported that complete removal of the corneal epithelium is an essential component of riboflavin-UVA crosslinking therapy as superficial epithelial trauma and tetracaine administration alone are not sufficient to permit the penetration of riboflavin into the corneal stroma. [106]

Samaras KE and O'Brart compared stromal riboflavin absorption after 20% alcohol application and partial or complete epithelial removal using a spectrophotometer, they found that complete removal of the corneal epithelium appears to be necessary to allow sufficient riboflavin absorption into the stroma.[105]

Touboul et al compared conventional, transepithelial, and accelerated CXL in 24 patients. Confocal microscopy showed corneal changes in conventional and accelerated crosslinking (ie, when the epithelium was removed), but in TCXL, there were no observable changes in the cornea. They concluded that TCXL had no benefit. [144]

As regards the 2 eyes which undergone epithelial scratches the demarcation line was detected at a depth of about 200 microns and this does not correspond with the findings of other researches that found that even if the tight junctions between superficial epithelial cells are removed with an excimer laser, the basal epithelial cell layers act as a barrier to riboflavin penetration.[145] Similarly,Alhamad et al found that superficial scraping with a thin needle, creating a grid pattern, was found to be insufficient to allow riboflavin penetration to the stroma.[146]

In group IIB (epi-on, Ricrolin), the demarcation line was observed at a depth of about 200 μm(mean DLD at central location was 198 μm). Filippello et al reported a demarcation line 2 weeks after TCXL that was located approximately 100 μm from the corneal epithelium using confocal microscopy Transepithelial CXL was performed by applying an enhanced riboflavin solution (riboflavin 0.1%, dextrane T500 with trometamol

[Tris-hydroxymethyl aminomethane] and EDTA [ethylenediaminetetraacetic] sodium salt) on the intact corneal epithelium for 30 minutes before irradiation with ultraviolet A (370 nm at 3 mW/cm^2) for 30 minutes. [147,148]

More recent studies have shown support for the efficacy of TCXL. Pinelli and colleagues reported no significant difference between TCXL and standard CXL. [149]

Stojanovic et al evaluated the efficacy and safety of TCXL using a multifactorial approach to achieve proper stromal riboflavin saturation. The authors used a non– -dextran-containing, hypotonic solution and employed superficial disruption of the epithelial surface using Merocel sponge in the conjunctival sac in order to enhance penetration of riboflavin. Riboflavin saturation was confirmed via slit lamp, rather than using an arbitrary time limit, the mean demarcation line was located at the depth of 317 μm from the surface. [150]

The line was significantly deeper in epi-off group than that in the group treated with ricrolin TE at central and temporal location at 1 mm, while there were no statistically significant difference between the two groups demarcation line depth to corneal thickness ratio at all locations. It is indeed this limited penetration of TE CXL that allows thinner corneas (between 360 and 400 μm) to be treated, posing no risk to the endothelium.

An essential requirement for CXL is obtaining an adequate concentration of riboflavin within the corneal stroma. In the late 1990s, the first clinical studies of CXL were performed by a group in Dresden led by Theo Seiler, MD, PhD The photosensitizer used at that time was 0.1% isotonic riboflavin solution in 20% dextran. This formulation had limited ability to penetrate the intact epithelium due to the large molecular sizes of riboflavin and dextran.. The Dresden protocol therefore required epithelial removal in order to allow better penetration of the riboflavin preparation. [82,151]

In most of the studies against transepithelial CXL, the standard riboflavin formulations were used. For this reason, newer formulations of riboflavin such as Ricrolin TE have been formulated using alternative vehicles (amino alcohol TRIS (trometamol and the EDTA disodium salt), which, in combination, enable transepithelial penetration.

Pentacam results:

- **Keratometry readings:**

Regarding keratometry readings, we illustrated that there was no statistically significant difference between preoperative and postoperative K1 and K2 values in the three studied groups.

There was no statistically significant difference between the three studied groups according to K1and K 2 change and these results were comparable to other published CXL studies.

Stojanovic et al reported non significant alteration regarding Mean-K, while the Max-K showed a significant decrease (P = 0.02) at the location of the cone. [150]

El-Raggal etal reported a statistically significant decrease in K readings after three months in epi-off CXL. [93]

Vinciguerra et al found Initial worsening of all keratoconus indices, a dramatic change observed in corneal power with an increase in steepest meridian keratometry, and simulated cylinder values at 1 month after CXL (probably because of epithelial debridement)there was a slow but continuous improvement of the indices up to 12 months postoperatively. [92,94]

The epithelium acts as a smoothing agent that reduces corneal power, astigmatism, and irregularity of keratoconic corneas. [152,153,154] This finding explains why topography obtained 1 month after epi-off CXL paradoxically shows an increase in the steepness of the cone. Instead of an effect of the CXL procedure itself, the increase results from the epithelial debridement alone.

- **Pachymetry:**

Regarding pachymetry values there was a statistical significant decrease in postoperative pachymetry at corneal thinnest location in group I, while in group IIA and IIB no statistical significant difference existed between pre operative and postoperative pachymetry.There was a statistically significant difference between group I and IIA at $p \leq 0.05$as regards preoperative and postoperative thinnest location change, and between group I and IIB at $p \leq 0.01$ and these findings were similar to results reported by Stojanovic et al that showed a significant decrease in thickness at 1-month follow up. [150]

Koller et al found using schiempflug camera at the 1-month postoperative examination, a significant reduction of the pachymetry at the thinnest point by approximately 50 µm on average[155], which is not in agreement with the results of Caporossi et al who reported no significant reduction of the pachymetry after epi off CXL.[90]

Using ultrasound pachymetry, the Siena Group reports a statistically nonsignificant increase in central corneal thickness by 30 µm at 1 month after epi-off CXL treatment, slightly reducing to 20 µm at 3 months. The authors interpret this increase as corneal edema that clears with time. [125] However, measuring corneal thickness in keratoconus corneas by means of ultrasound pachymetry seems to be questionable because of various reasons. [156]

Ucakhan et al found a significant smaller central corneal thickness measured with the pentacam compared with ultrasound pachymetry and a relatively poor correlation between the 2 techniques. During the early postoperative phase, one could discuss the reduced corneal thickness as a measurement artifact because of the haze seen in almost all patients.[157]

There are several ways to evaluate the effect of CXL, corneal topography changes, pentacam findings including pachymetry, and demarcation line detection .[94,144,147] It is important to realize that a flattening effect of a keratoconic cornea has never before been documented.

Although the precise nature and significance of the demarcation line in relation to the cross-linking process are uncertain, but it usually coincides with the topographic changes (post-operative regression) which was found in 70 % of CXL patients. [124]

SUMMARY

Keratoconus Is a progressive, noninflammatory, bilateral (but usually asymmetrical) ectatic corneal disease, characterized by paraxial stromal thinning and weakening that leads to corneal surface distortion.Corneal topography and to a great extent pentacam, aid in the diagnosis of keratoconus.

Depending on the degree of keratoconus, treatment aims at two main entities:

A-Refractive error correction using spectacles or contact lenses(soft or hard) in early cases progressing to keratoplasty either penetrating keratoplasty or deep anterior lamellar keratoplasty in advanced KC.

B-Stabilization of the disease using corneal collagen cross-linking (CXL):

- Epithelium off CXL is the standard protocol recommended removal of the epithelium before impregnation of the cornea with a diffusible photosensitizer (riboflavin) and UVA irradiation.
- Transepithelial CXL is a modification of the standard technique by keeping the epithelium intact.

Stromal demarcation line indicating the transition zone between cross-linked anterior corneal stroma and untreated posterior corneal stroma can be detected using AS-OCT. The presence of this finding over the anterior two-thirds of the stroma confirms that sufficient CXL treatment has occurred.

The aim of work is to compare the demarcation line depth following epithelium off and transepithelial corneal collagen cross linking in keratoconic patients.

Our study was conducted on 40 eyes of 20 keratoconus patients with clear central cornea.Group I includes 20 eyes undergone epithelium off CXL with age range between 15 and 31 years with mean =23.10 ± 5.45, group IIA includes 10 eyes undergone transepithelial CXL using Medio Cross TE-Transepithelial solution and CCL-HE machine with age range between 13 and 29 years with mean =20.60 ± 6.07, and group IIB includes 10 eyes undergone transepithelial CXL using Ricrolin TE and CCL-365 machine with age range between 20 and 35 years with mean =29.20 ± 7.16.

Scheimpflug camera imaging was done to all patients preoperatively and postoperatively after one month, postoperative AS-OCT was done to all cases after one month.

Our results revealed that:

- No statistically different findings between group I and group IIB corneal stromal demarcation line depth at both nasal locations and temporal location at 2 mm.
- Mean stromal demarcation line depth of the central and temporal location at 1 mm in group I was significantly deeper than in group IIB.
- Corneal stromal demarcation line depth to corneal thickness ratio shows no significant different between the two groups.

- In group IIA the line was not detected except in 2 eyes which undergone epithelial scratches.
- There was no statistically significant difference between the three studied groups according to K1 and K2 change.
- There was statistically significant difference between group I and IIA at $p \leq 0.05$as regards preoperative and postoperative thinnest location change, and between group I and IIB at $p \leq 0.01$.

Table X summarizes the difference between epithelium off and epithelium on CXL.

Table X : Differences between epithelium off and epithelium on CXL.

	Epithelium off	Epithelium on
Technique	The standard technique	A modification of the standard technique
Epithelium	Preceded by removal of the epithelium	The epithelium is kept intact.
Riboflavin	0.1% isotonic riboflavin solution in 20% dextran	Group IIA:Mediocross TE Group IIB:Ricrolin TE+enhancer
Corneal thickness	Used in corneas thicker than 400 microns	Thinner corneas could be used
Healing of epithelium	Needs time	Epithelium is kept intact
Risk of infection	Higher	Less
Demarcation line depth	Deeper	Absent or more superficial
Pain	More	Less
PRK	Could be done	Could not be done