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Comparison of Corneal Stromal Demarcation Line Depth in Epi-off and Epi-on Corneal Cross-Linking Using Anterior Segment Optical Coherence Tomography

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ABSTRACT

Objective: To compare the corneal stromal demarcation line depth after Epithelium-On and Epithelium-Off corneal cross-linking in patients with keratoconus using anterior segment Optical Coherence Tomography (AS-OCT).
Study design. Quasi-experimental study.
Place and Duration of Study: Armed Forces Institute of Ophthalmology, Rawalpindi Pakistan from Jun 2018 to Jun 2019.
Methodology. This study included patients (60 eyes) with progressive keratoconus treated with procedure CXL (corneal cross linkage). There were 30 eyes in each group. Two different protocols of Corneal cross linkage were performed, and patients were divided into two groups according to protocol: 1) epithelium-on Corneal cross-linkage, 2) epithelium-off Corneal cross-linkage. In the First-Group, epithelium-on Corneal cross-linkage, corneal epithelium was not removed, riboflavin solution was instilled on the cornea for one hour, and the cornea was exposed to Ultraviolet A rays. In Epithelium off Corneal cross-linkage, after epithelium was removed by spatula, riboflavin solution was instilled for 30 minutes, and Ultraviolet-A rays were focused on the cornea. After one month of the procedure, Anterior segment Optical Coherence Tomography was performed. Demarcation line depth was measured in treated corneas in all patients from both groups, and findings were compared.
Results. The stromal demarcation line depth was similar in both groups, and there was no statistical difference between the groups. The mean value of demarcation line depth was 277.13±107.09 µm (p>0.05).
Conclusion. Corneal stromal demarcation line depth after both procedures was similar in both groups after one month of Corneal cross-linkage, so it was concluded that epithelium-on Corneal cross-linkage was comparable to epithelium-off Corneal cross-linkage.

Keywords: Cross-linking, Demarcation line, Keratoconus, Optical Coherence Tomography

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INTRODUCTION

Keratoconus is a non-inflammatory and asymmetrical disease involving the cornea of both eyes in a patient.[1] The cornea with keratoconus is more elastic, has less rigidity,[2] and is characterized by progressive corneal ectasialeading to not only reduction in biomechanical stability of cornea but also impairing the visual acuity due to irregular astigmatism, myopia and corneal scarring.[3,4]

Different treatment options for keratoconus are available for patients, like a hard contact lens, intracorneal stromal ring(ICSR), CXL and penetrating keratoplasty, but only CXL is the treatment which can stop the progression of the disease.[5] Studies support that corneal cross-linking (CXL) preserves the cornea and improves its biomechanical strength,[1] and has reduced the number of patients requiring corneal transplant.[6]

In corneal cross-linking, UVA (370nm) activates riboflavin (Vit B2) which in an excited triplet state omit reactive oxygen radicals,[1] causing formation of bonds in collagen fibrils,[6] without losing corneal transparency & improves vision to some extent & halt progression.[7]

One of the newer protocols is Epi-on CXL, also called transepithelial CXL which is gaining popularity as it is simpler and less invasive than epi-off CXL. There is lesser discomfort and decreased,[8] corneal haze seen after procedure,[2] but its efficacy is still debated internationally as corneal epithelium reduces drug penetration and causes uneven distribution of the drug in stroma.[6]

The parameters for the efficacy of CXL are being evaluated, and the corneal demarcation line is considered reliable. The histopathological evaluation of cornea after undergoing CXL have shown keratocytes apoptosis restricted to anterior stroma just after 24 hours with a higher density of extracellular matrix in stroma.[9] This line demarcates the transition between treated anterior part and posterior non crossed linked cornea. The effective depth can be measured with the help of different instruments like confocal microscopy,

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ultrasound and anterior segment optical coherence tomography.[10]

The objective of the study was to compare the efficacy of epi-on and epi-off CXL taking demarcation line depth as a parameter. Epi-off CXL has been performed for many years and is a standard procedure. In contrast, Epi-on CXL is emerging as an alternative, but as very little work is being documented regarding epi-on CXL, its efficacy and safety are not being completely established yet. Hence this study was performed, and results were compared after both techniques taking demarcation line depth as a parameter.

METHODOLOGY

This quasi-experimental study was carried out at the Armed Forces Institute of Ophthalmology, Rawal-+pindi Pakistan, from June 2018 to June 2019 after approval of the Hospital Ethical Review Board (Reference Number: 203/ERC/AFIO) adhering to the tenets of the Declaration of Helsinki. The sample size was calculated by the WHO sample size calculator by keeping 95% CI, 5% margin of error, mean demarcation line depth for Group-1 C-CXL 275.05±41.8 um and Group-2 TE-CXL 132.60±22.14 um.[10] Non-probability consecutive sampling technique was used. All patients included were informed about the risks and benefits of the procedure, and consent was taken.

Inclusion Criteria: The study included participants with progressive keratoconus with no other ocular or systemic disease. The inclusion criteria were the progression of 1D of astigmatism in the last year with deterioration in vision.

Exclusion Criteria: Patients with active ocular infection, previous herpetic eye disease, previous ocular surgery, diabetes, pregnancy, lactation were excluded from the study.

A thorough history was taken, including age, gender, disease duration, family history, history of ocular allergy, and contact lens wear. A detailed examination was carried out, including Visual assessment, BCVA, and slit lamp examination to view the anterior and posterior segments. Scheimpflung topography was performed using Galilei G4 and the thinnest pachymetry, topographic astigmatism, mean K, and maximum K was recorded.

Based on corneal pachymetry, participants were assigned into two groups. Patients with corneal thickness lesser than 400 um at the thinnest point were enrolled in (1) epi on the CXL Group, whereas eyes with pachymetric values greater than 400um at the thinnest point were included in (2) epi off the CXL Group.

All the procedures were performed as day-care surgery under aseptic conditions in the operation theatre. Pre-operatively topical anaesthesia was instilled. Alid speculum was inserted. In the epi-off CXL procedure, a central 7-9mm of the epithelium was removed mechanically with a blunt metal spatula, isotonic solution of Riboflavin 0.1%, HPMC 1% was instilled every two minutes for 30 minutes, and after confirming penetration of riboflavin in stroma by a flare on slit lamp, the cornea was exposed to UVA light with an irradiance of 9mW/cm² for 10mins at distance of 5cm.

In epi-on CXL, the epithelium was not removed, and hypotonic saline of 0.25% riboflavin with benzalkonium chloride was instilled every 2 minutes for 1 hour. Then anterior chamber flare was checked on a slit lamp to confirm complete penetration of riboflavin in the cornea, and UVA irradiation was applied at an irradiance of 9mW/cm². Riboflavin was instilled every 3 minutes after that for 30 mins during UVA irradiation.

After CXL was performed patient's eye in both techniques was rinsed with a balanced salt solution, a therapeutic bandage contact lens was applied, and topical antibiotics and cycloplegics were advised. Patients were followed up after one week and then fortnightly. During each visit, VA, BCVA and slit lamp examination was performed, and on the third visit, anterior segment OCT was performed for demarcation line depth measurement.

In AS-OCT (3D OCT-2000 TOPCON), corneal images were taken when corneal reflex was seen, and stromal demarcation line depth is measured from corneal epithelium to hyperreflective line into the stroma as shown in Figures-1 & 2.

Statistical Package for Social Sciences (SPSS) version 20:00 was used for the data analysis. Quantitative variables were summarized as Mean±SD. Independent sample t-test was applied to find the mean differences among the groups. The p-value lower than or up to 0.05 was considered as significant.

RESULTS

A total of 60 eyes of 60 patients who were found to have progressive keratoconus and required corneal cross-linking were included in the study. Thirty eyes

had epi-on CXL, and 30 eyes had epi-off CXL. The mean age in both groups was 14.96±3.73 years. The mean of demarcation line depth in both was 277.13±107.09 µm. No statistical difference was found (p=1.00) in demarcation line depth in both groups.

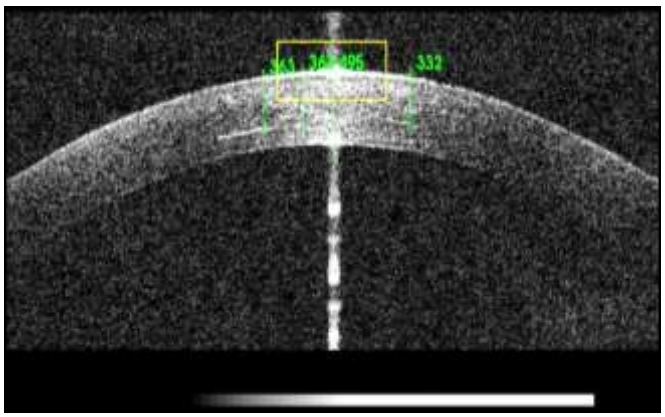

Figure-1: High Resolution AS-OCT (Anterior Segment Optical Coherence Tomography) Scan Showing Demarcation Line after One Month of Epi-Off CXL

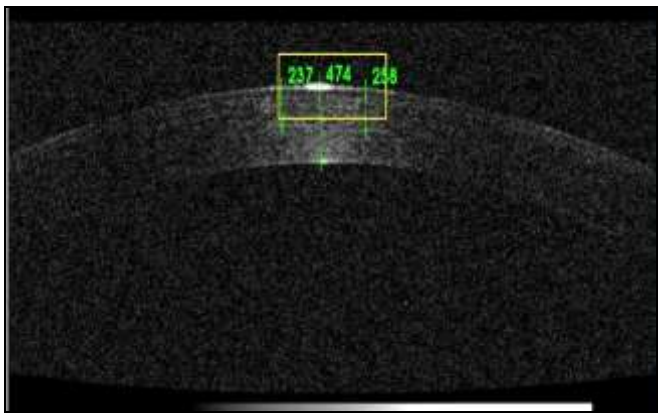

Figure-2: AS-OCT Showing Demarcation Line In Epi -On CXL after One Month of Treatment

The mean K1 in both groups was 45.58±2.43D with no significant difference (p=1.00) of K1 in epi-on and epi-off. The mean K2 in both group was 48.26± 3.30D with no significant difference (p =1.00) of K2 in epi-on and epi-off. The minimum corneal thickness measured through Scheimpflug corneal tomography in patients was 360um.

The mean of both groups was 464.16±49.22 µm, with no statistically significant minimum corneal thickness in both group (p=1.00), as CCT(central corneal thickness) was also not significant (p=1.00) of both groups' mean of CCT in epi-on and epi-off was 481.83± 41.02 µm as shown in Table.

Table: Comparison of Parameters in Both Groups (n=60)

Parameters	Epithelium on (Epi-on) n=30	Epithelium off (Epi-off) n=30	*p*-value
	Mean±SD		
Depth (µm)	277.13±107.09	277.13±107.09	1.00
Flat Keratometery K1(D)	45.58±2.43	45.58±2.43	1.00
Steep keratometery K2(D)	48.26±3.30	48.26±3.30	1.00
Minimum corneal thickness µm)	464.16±49.22	464.16±49.22	1.00
Central corneal thickness (CCT µm)	481.83± 41.02	481.83±41.02	1.00

DISCUSSION

In this study, we compared standard epi-off CXL with epi-on CXL procedure, taking demarcation line depth as a parameter and measuring the stromal demarcation line depth in the cornea using AS-OCT. We observed similar depth in both groups. The conventional epi-off CXL known as Dresden protocol is already known for its safety and efficacy in halting the progression of keratoconus.[11] We compared this conventional technique with a newer epi on CXL and observed the same treatment effect. Similar to our study Cifariello *et al*. concluded in their research work that both epi-on and epi-off techniques slow down the progression of disease equally. He also concluded in this study that epi-on CXL is preferable to epi-off CXL as it not only maintains the corneal thickness but also causes patients to experience lesser post-procedure discomfort and more improvement in visual acuity.[12]

Another study which was conducted by Amir *et al*. concluded that there is no significant difference between epi-off and epi-on collagen cross-linkage procedures in terms of visual outcome and topographical parameters, but rather there was the improvement in mean topographic astigmatism in epi-on CXL as compared to other groups which render it an as a superior technique when compared to conventional CXL[13].

Since the start of CXL, different parameters have been debated for assessing the effectiveness of the procedure. Peyman et al. believed, that depth of demarcation line in corneal stroma is a reliable indirect measure of CXL efficacy, but still, much understanding is needed about changes in corneal stroma after CXL.[14] There are studies that have shown shallower demarcation line depth in epi-on CXL when compared with epi-off.

CXL attributed this difference to the shielding effect of intact epithelium in this epi-on technique.[10] As Bikbova *et al*. have reported, there was superficially cross-linking in the stroma, so demarcation line depth is shallower in transepithelial iontophoresis–assisted CXL rendering it less effective as compared to standard CXL protocol, but disease progression was stopped in both techniques after 24 months.[15]

Similarly, Abdel-Radi *et al*. compared corneal demarcation line depth in epithelium off and epithelium on accelerated CXL. The Demarcation line depth in Group A was 219.9±58.4 um, and in Group B, it was 127.2±7.8 (p<0.05). They also recorded the correlation of (maximum keratometry) Kmax with keratoconus progression in both techniques and concluded that although there is a significant difference in demarcation line depth in both groups, there is no significant difference in keratoconus progression.[16] Salah *et al*. included 40 eyes of 20 patients and performed transepithelial accelerated CXL in one eye, and epithelium-off accelerated cross-linking in the other eye of each patient and concluded that the demarcation line was more defined and deeper in the epi-off accelerated cross-linking. [17]

Stulting *et al*. reported that visual improvement in epi-on CXL is similar to epi-off CXL and suggested not only that it can be repeated safely in eyes where the ecstatic process continues but that increased safety and rapid recovery proves this technique superior to epi-off CXL for keratoconus.[18] Whereas Godefrooll *et al*. reported no superiority of trans-CXL over epi-off CXL regarding visual high-order aberrations(HOA) as there was no difference in HOAs between the groups after treatment.[19]

Previously only a standard technique that is epi off CXL was performed. It had some complications like severe pain, reactivation of herpetic keratitis and central corneal scarring.[20] There is ongoing research to establish the efficacy of techniques like epi-on CXL in patients with keratoconus. It can help an ophthalmologist choose an appropriate technique for a selected case, especially patients with thinner corneas, as Bikbova *et al*. has reported stabilization of disease for 24 months in epi-on CXL and preferred technique in patients with thin cornea and patients intolerant to pain.[15]

Every modified approach of CXL has to be compared to standard CXL due to its long-duration validity, and we have done the same in our study. Until now, no specified amount mentioned in the literature regarding CXL that how much stromal tissue cross-linking is required to consider CXL effective,[11] so there is no set parameter to evaluate CXL's definite success. Further studies are required in larger population groups and with longer follow-up duration to validate the results further.

CONCLUSION

Corneal stromal demarcation line depth after both procedures was similar in both groups after one month of Corneal cross-linkage, so the newer technique epithelium-on corneal cross linkage was comparable to standard epithelium-off Corneal cross linkage taking stromal demarcation line depth as a parameter.

Conflict of Intrest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

QA: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

OZ: Conception, Study design, drafting the manuscript, approval of the final version to be published.

IB & MA**:** Drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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