Research Article

Evaluation of corneal endothelium cell density pre and post Lasik with accelerated cross-linking

Mohamed S. Tohamy, Ahmed M. Eid,
Ahmed M. Sabry and Mohamed S. Mahmoud
Department of Ophthalmology, Minia University

Abstract

Background: Myopia is a common refractive error all over the world. Many methods can be used for correction of myopia. LASIK has become one of the most popular procedures for the reduction or elimination of myopic refractive errors and has emerged as the refractive corneal surgical procedure of choice for the correction of myopia, in correcting moderate to high myopia (equal or more than -6.00 D in the least-minus meridian). Significant long-term regression and Post LASIK ectasia was the motivation behind attempting to apply prophylactic in situ corneal collagen cross-linking (CXL) on the stromal bed, concurrently with the LASIK procedure, particularly in high-myopic eyes with thin residual stroma and in younger patients who may not yet have exhibited ectasia risk factors. Corneal endothelium is a mono layer of the cornea that maintains corneal deturgescence and transparency through regulating fluid and solute transport between the aqueous and corneal stroma. Endothelial cell analysis is one of the methods that is used to assess the health of the cornea, especially after surgical intervention and is also one of the major criteria that are used to assess the long-term outcomes of any corneal surgery. **The aim of the study** is to assess the safety of CXL on the corneal endothelium density by endothelial specular microscopy before and after the procedure. **Patients and methods:** This prospective study was included 30 myopic patients (7 male and 23 female, 60 eyes) with range of age from 18 to 35 years. Patients were divided into two groups, Group A Included 30 eyes of 15 patients, treated by LASIK, Group B Included 30 eyes of 15 patients, treated by LASIK associated with accelerated CXL. **Results:** Qualitative and quantitative analysis of the corneal endothelial cells comparing the two groups showed a statistically significant changes in ECD (P=0.040) 3 months follow up after the procedure which improved to reach a value close to preoperative values with no significant changes between the two groups at 6 months follow up (P=0.081), there were no significant changes in evaluating CV (polymegathism) and percentage of hexagonal cells (pleomorphism) in each group and in comparing between the two groups 3 and 6 months after follow up. **Conclusion:** Specular microscopy is a non-invasive fast technique for evaluation of corneal endothelium Lasik with accelerated CXL is a new, innovative procedure, aimed at reducing some of the challenges associated with LASIK (weaker corneas, and regression of the refraction over time). This study revealed that LASIK with accelerated CXL (30 mW/cm^2 for 90 seconds) is safe and has no toxic effect on corneal endothelium.

Keywords: Myopia, corneal endothelium cell, polymegathism

Introduction

Corneal endothelium plays an important role in maintaining corneal transparency. A decline in endothelial cell density (ECD) may reflect a decrease in the ability to pump fluid out of the cornea, leading to corneal edema. ECD is a key quantitative corneal endothelial parameter for the evaluation of corneal health. Accurate ECD measurements and image quality are important in clinical practice,[(1)] and is also one of the major criteria that are used to assess the long-term outcomes of any corneal surgery.[(2)]

Laser-assisted in situ keratomileusis (LASIK) is the most common form of refractive surgery, offering predictable and stable refractive and visual outcomes. Specifically, in correcting moderate to high myopia (equal or more than -

6.00 D in the least-minus meridian),[3] there have been reports in the past indicating significant long-term regression, 20.8% of high myopia cases required retreatment because of over- or under correction, or regression.[4] Other studies have shown that the risk of regression may be between 5% and 27%.[5] This was the motivation behind attempting to apply prophylactic in situ corneal collagen cross-linking (CXL) on the stromal bed, concurrently with the LASIK procedure, particularly in high-myopic eyes with thin residual stroma and in younger patients who may not yet have exhibited ectasia risk factors. The application aims to enhance corneal rigidity and thus reduce the possibility of long-term myopic shift.[6, 7]

Patients and Methods

Study design:

The study was a prospective comparative interventional case series. It was conducted in International Eye Center and Roaa Laser Vision Correction Center from January 2018 to January 2019.

Patients:

This prospective study was included 30 myopic patients (7 male and 23 female, 60 eyes) with range of age from 18 to 35 years.

Patients were divided into two groups:

Group A: Included 30 eyes of 15 patients, treated by LASIK.

Group B: Included 30 eyes of 15 patients, treated by LASIK associated with accelerated CXL.

Ethical consideration:

- All patients included in this study were informed about the details, risks, and nature of the study.
- An informed written consent to be involved in the study was obtained from each patient.
- Approval of the ethical committee of the faculty of medicine El- Minia university was obtained.

Inclusion criteria:

- Age: from 18 to 35 years.
- Spherical Myopia: from -3 Dioptre (D) to -8 D and astigmatism of less Than -5 D.
- Minimal pachymetry: 480 µm.
- K-reading: from 41 D to 48 D.

Exclusion criteria:

- Previous history of ocular surgery.
- History of systemic diseases e.g. Diabetes Mellitus (DM), hypertension, autoimmune patients.
- Myopia more than -8 D.
- Keratoconus.
- Corneal thickness less than 480 µm and predicted post-operative residual stromal bed thickness of less than 280 µm.
- Hyperopic patient.
- Ocular media opacity e.g. cataract, corneal opacity, glaucoma.
- Recent contact-lens users.
- Pregnancy or lactation.
- Uncooperative patient.

Preoperative evaluation:

I. History:

1. Systemic:

a) Patients were asked about their previous general medical history as DM.

b) Female patients were asked about their status regarding pregnancy, breast feeding, or the use of oral contraceptive drugs or the use of hormone replacement therapy.

2. Ocular:

a) Patients were asked about their ocular history regarding medical and surgical ophthalmic history and the previous use of contact lenses, trauma, and use of eye drops.

b) History of use of spectacles and changes in the previous prescriptions in the past year.

II. Examination:

1. Visual acuity (V/A): was measured by snellen's chart, manifest and cycloplegic refraction was also measured.

2. slit-lamp examination:

a) Searching for signs of dry eye and tear film assessment (tear meniscus and breakup time), detailed examination of the cornea to rule out undiagnosed corneal dystrophies, allergic conjunctivitis, other pathologies of the conjunctiva, sclera, lens and iris

b) Intraocular pressure was measured using Goldmann applanation, to exclude glaucoma.

3. Fundus examination:

Detailed examination by to reveal signs of diabetic retinopathy, maculopathy or optic nerve disease. Examining the periphery to exclude retinal detachment or peripheral retinal lesions by indirect ophthalmoscope, which may increase the risk of retinal detachment.

III. Investigation:

1- Rotating scheimpflug camera and placido disc (Sirius, CSO, Italy) to assess corneal

topography and corneal thickness before the procedure.

2- Specular microscopy (Tomey, EM-3000, Japan) Will be done three times; 1st base-line (before the procedure), at three and six months after procedure.

Surgical steps:

- All cases were operated upon using the **(Abbott Star S4 IR)** excimer laser.
- All procedures were performed first on the right eye, then on the left eye, using the same blade.
- The cornea was marked with a standard LASIK marker.
- For the right eye the incision was made from the temporal and inferior side to the nasal side. For the left eye, the incision was made from the nasal side to the temporal side.
- The suction ring was applied. The micro-keratome M-2® microkeratome (Moria, France) was then placed over the dovetail, and locked into place.
- The M-2® microkeratome (Moria, France) was used to create the flap, the blade was then advanced forward, then backwards, and the suction was released.
- The microkeratome was removed, and the flap reflected, drying the stromal bed and the stromal ablation was applied conventional laser.
- The ablated bed was irrigated.
- The flap was returned into place, the interface was irrigated again, and the flap was repositioned according to the alignment marks, and allowed to dry for two minutes. Topical antibiotics and steroids were applied

At group B of patient

- 0.25% Riboflavin in 20% hydroxymethyl propyl cellulose solution (vibex rapid, Avedro inc, USA) was instilled topically every 30 seconds for 90 seconds at the stromal bed before the flap returned into its place after the ablation.
- Stromal bed irrigated to remove riboflavin and returning the flap to its position according to the alignment marks. The cornea was exposed to UV-A light of 366-374 nm at an irradiance of 30 mW/cm^2 for 90 seconds (OMNI, MMD, USA) with total energy (2.7j/ cm^2)

Results

The study included sixty eyes allocated in two groups:

Group A: included 30 eyes of 15 patients treated with LASIK, 4 males and 11 females. The mean age was 26.8±3.7 years.

Group B: included 30 eyes of 15 patients treated with LASIK with CXL, 3 males and 12 females. The mean age was 27.9±5.3 years.

All patients were evaluated 3 and 6 month after the procedure.

Table (1): Demographic and clinical characteristics of the studied individuals.

		Group I N=30	Group II N=30	P value
Age	*Range* *Mean ± SD*	(18-34) 26.8±3.7	(19-35) 27.9±5.3	0.371
Sex	*Male* *Female*	8(26.7%) 22(73.3%)	6(20%) 24(80%)	0.542

- Independent samples T test for quantitative data between the two groups.
- Chi square test for qualitative data between the two groups.
- *: Significant difference at P value < 0.05

In patients treated with LASIK, the mean endothelial cell density was 2793.7±115.7 CD/mm^2 preoperative (table 3). 3 months after the procedure was 2754.3±101.6 CD/mm^2 (p value=0.001), and after 6 months was 2782.7± 107.2 CD/mm^2 (p value=0.088). The difference between the 3 months and 6 months postoperative values was significant at P value=0.001.

On the other hand patients treated by LASIK with CXL, the mean endothelial cell density

was 2745.8±199.7 CD/mm^2 preoperative. Three months after the procedure was 2647.1±257.3 CD/mm^2 (p value=*0*.005), and after six months was 2696.3±241.9 CD/mm^2 (p value=*0.146*). The difference between the 3 months and 6 months postoperative values was significant difference (P=0.005).

In comparing between the two group, no significant difference (p=0.261) preoperatively, 3 months after the procedure was significant difference (p=0.0040), at 6 months of follow up (p=0.081) with no significant difference between the two groups.

Table 2 endothelial cell count (CD/mm^2) pre and postoperative with P values in the two group.

CD/mm^2		**Group I**	**Group II**	**P value**
		N=30	**N=30**	
Preoperative	*Range* *Mean ± SD*	(2582-2993) 2793.7±115.7	(2427-3177) 2745.8±199.7	*0.261*
3 months postoperative	*Range* *Mean ± SD*	(2500-2890) 2754.3±101.6	(1898-3122) 2647.1±257.3	***0.040******
6 months postoperative	*Range* *Mean ± SD*	(2591-2975) 2782.7±107.2	(1969-3240) 2696.3±241.9	*0.081*
P value				
Pre vs 3 months		***0.001****	***0.005****	
Pre vs 6 months		*0.088*	*0.146*	
3 months vs 6 months		***0.001****	***0.005****	

- Independent samples T test for quantitative data between the two groups.
- Paired samples T test for quantitative data between each two times within each group.
- *: Significant difference at P value < 0.05

Discussion

The development of progressive postoperative LASIK keratectasia is rare but visually debilitating to the patient. LASIK is challenging in patients with high myopia due to the higher risk of developing post-operative keratectasia and refractive regression. The difficulty of determining who is at risk of postoperative LASIK keratectasia coupled with the regression of LASIK correction over time, particularly in younger patients and those with high myopic corrections, makes it a major challenge.[5, 97] Hence, the use of simultaneous accelerated CXL and LASIK to stabilize the patient's refraction and cornea immediately after LASIK may be useful, particularly in these high-risk patients. One considerable limitation of accelerated CXL is its impact on the corneal endothelium. Several previous studies Kymionis GD, et al.,., and Gokhale NS. have reported contradictory results on corneal endothelial cells after using standard CXL.[98, 99]

However, only few published clinical studies are currently available which evaluate the effects of accelerated CXL on the corneal endothelium by calculating different parameters. [100, 101] This study evaluates the effects of accelerated CXL on corneal endothelium density, the percentage of hexagonal cells, and CV. Standard CXL irradiance protocols use UV-A energy of 3 mW/cm2 for 30 minutes in association with the application of hydrophilic riboflavin 0.1%. This causes a considerable and relatively acute decline in UV-A light of up to 95% and therefore, a resultant irradiance of the endothelium was only 0.15 mW/cm2 (=0.27 J/cm2) with corneal thickness of >500 µm. [102] In order to reduce the exposure time of the procedure, According to the Bunsen–Roscoe law of reciprocity, a higher intensity of the UV-A is required, with a similar overall effect and similar sub-threshold cytotoxic endothelial UV-A dosage.[103] Consequently, treatment at 3 mW/cm2 for 30 minutes is equivalent to that at 9 mW/cm2 for 10 minutes, 18 mW/cm2 for 5 minutes and 30 mW/cm2 for 3 minutes.[104] Current treatment protocols use UV-A energy at an irradiance of 30 mW/cm2 for 90 seconds and at a 5 cm distance from the cornea, with an application of 0.1% riboflavin, saline with

hydroxyl propyl methylcellulose solution every 30 seconds for 2 minutes before irradiation and one drop every 30 seconds during the irradiation. Now, there are different commercial devices with ultrafast settings such as 43 mW/cm2 for 2 minutes. Despite the availability of all of these settings, still verification of clinical efficacy and safety are not possible for further studies.[104]A potential risk of CXL is that it may induce endothelial cell damage. The ultraviolet irradiation energy of the traditional CXL procedure is 5.4J/cm2, which is far lower than the threshold to induce corneal endothelial cell loss, iris, crystalline lens, and or retinal damage,[105] although riboflavin saturation in the residual stromal bed in the LASIK with CXL treatment is relatively thin, the ultraviolet irradiation energy is only 2.7J/cm2, which is also lower than the threshold for corneal endothelial cell damage. Compared with the preoperative values, the ECD of LASIK with CXL group after 3 months showed statistical significant decrease(P=0.005) no obvious change after 6 months (P=0.146), and with insignificant changes comparing both groups with each other (P=*0.081*), pointing out that the combined surgery has no significant effect on postoperative endothelial cells. Studies have evaluated the endothelium after refractive surgery or CXL, finding little or no change, with no clinically significant reduction in ECD.[71, 106] However, with all corneas having pachymetry of more than 400mm during CXL treatment and no statistically significant difference in the change in ECD between the LASIK-only group and the LASIK-CXL group being found.

The results of this study are consistent with the results of other recent studies, with no differences in the irradiance parameters. Ying Wu, et al.,. found insignificant endothelial cell changes in both density (ECD) and morphology (CV and percentage of hexagonal cells) following accelerated CXL 30 mW/cm2 for 90 seconds (total exposure dose of 2.7J/cm2), [107] in our study and after examining and comparing CV (polymegathism) between the two groups, there were no significant changes; preoperatively (P=0.710), after 3 months (P=0.945) and 6 months (p=0.837) after the procedure.

Also our results in evaluating percentage of hexagonal cells (pleomorphism) pre and post operatively (3 and 6 months follow up) and in comparing between the two groups there is no significant difference pre and postoperative at 3 and 6 months, preoperative (P=0.458), 3 months postoperative (P=0.256) and 6 months postoperative (P=0.733).In other studies using CXL in treatment of keratoconus and LASIK ectasia, First, Cingü, et al.,. found significant endothelial cell changes in both density (ECD) and morphology (CV and percentage of hexagonal cells) following accelerated CXL (18 mW/cm2 for 5 minutes). The substantial obvious changes were observed at the first week and the first month, and then, corneal endothelial count returned to the baseline values at 6 months, whereas percentage of hexagonal cells and CV returned to their base values only at 3 months,[108]and those were the same results achieved by Amani E Badawy[102] ,The second study used more intensive UVA irradiance (30 mW/cm2 for 3 minutes) and reported that changes did not return to its base value. The statistically significant changes in ECD and CV were persistent until the end of 1-year follow-up.[109] In contrast, Cınar Y, et al.,. had assumed that accelerated CXL had inconsiderable impacts on the value of ECD and 6-month follow-up of 23 patients with progressive KC treated by accelerated CXL (9 mW/cm2 for 10 minutes) revealed insignificant endothelial cell changes (P=0.082).[110] Another comparative study by Kanellopoulos AJ between standard CXL (3 mW/cm2 for 30 minutes) in one eye of 21 patients and accelerated CXL (7 mW/cm2 for 15 minutes) in the fellow eye provided a similar result on the safety of both accelerated and standard CXL on the corneal endothelium.[111]The higher UV-A intensity radiation might lead to the damage of the nerve plexus and, thus, destroy the endothelial pump performance. The sub-basal nerve plexus secretes transported neuropeptides such as calcitonin-gene-related peptide and substance P, which play a supporting role in facilitating the transmission of signals through the Na/K-ATPase pumps in corneal endothelium.[112]

Corneal endothelial changes mean that the endothelium might be damaged but not totally lost. Therefore, the recovery occurred when the undamaged endothelium slides in and replaces the damaged endothelial cells then performs its function in the area of the damaged endothelium. The time by which the post CXL

healing process appeared to be complete, was known and it might overlap with the healing of photorefractive keratectomy.[113] The combination of low-risk profile and significant improvement in refractive stability supports LASIK-CXL as a promising adjunct to LASIK in reducing the likelihood of enhancement procedures, particularly in those patients with high dioptre corrections. Four studies that included a total of about 41,000 eyes have found that LASIK has an average retreatment rate of 12%. Most of these occur during the first 2 years after the LASIK procedure.[114-116] If the refractive results with Lasik-CXL are truly more stable, this should logically result in lower retreatment rates over time.

However, this study has a few limitations. It was a short-term follow-up study. It is not possible to determine the longitudinal change of LASIK-CXL treatment for myopia correction. The sample size of this study was relatively small and therefore the results should be interpreted cautiously. Further studies with a larger sample size and a longer term follow-up period are required to corroborate our results.

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