Corneal endothelial changes after laser-assisted in situ keratomileusis combined with high-fluence cross-linking

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Purpose
To evaluate corneal endothelial cells before and after laser-assisted in situ keratomileusis (LASIK) combined with accelerated, high-fluence collagen cross-linking (CXL) in myopic patients.
Patients and methods
In a prospective comparative nonrandomized interventional case series study, 60 myopic eyes of 30 patients (seven males and 23 females) with age ranged from 18 to 35 years were distributed into two groups. Group A included 30 eyes of 15 patients, treated by LASIK, whereas group B included 30 eyes of 15 patients treated by LASIK associated with high-fluence CXL. All patients were subjected to preoperative and 3- and 6-month postoperative evaluation of corneal endothelial profile using specular microscope.
Results
Qualitative and quantitative analysis of the corneal endothelial cells comparing the two groups showed statistically significant changes in endothelial cell density (P=0.040) at 3-month 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-month follow-up (P=0.081). There was no significant change in polymegathism or coefficient of variation and in the percentage of hexagonal cells (pleomorphism) in each group and in comparing between the two groups at 3- and 6-month follow-up.
Conclusions
LASIK with high-fluence CXL is safe and has no adverse effect on corneal endothelium.

Keywords:
collagen cross-linking, endothelial cell, laser-assisted in situ keratomileusis Xtra, laser-assisted in situ keratomileusis, myopia, specular microscope

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Introduction

Laser-assisted in situ keratomileusis (LASIK) surgery is one of the most commonly performed surgeries all over the world because of the precise and rapid improvement of visual acuity, the smooth postoperative recovery, and the obvious improvement of patients' quality of life [1,2].

Corneal collagen cross-linking (CXL) is a technique used to increase the biomechanical strength of the cornea through induction of additional cross-links between collagen fibers using ultraviolet-A (UV-A) light and riboflavin as photomediators. It has been considered the standard treatment of keratoconus (KC) and iatrogenic corneal ectasia [3]. Accelerated cross-linking using higher irradiance (30 mw/cm^2) has been proven to be effective when used for treatment of patients with KC or iatrogenic corneal ectasia. It is comparable to conventional CXL in stabilization of the cornea, with similar or better safety profile [4,5]. The combination of LASIK and CXL is aimed at restoring strength to the cornea, increasing the stability of the visual outcomes, and improving the accuracy of the refractive correction. This reduces the incidence of iatrogenic ectasia, treatment regression, and the need for enhancements [6]. LASIK Xtra is used mainly in patients with higher risk of post-LASIK regression including those with hyperopia [7], high grades of myopia, younger age, and those with borderline anticipated residual stromal bed thickness [8].

Specular microscopy is a noninvasive photographic technique that allows visualization and analysis of the corneal endothelium, which appears as a rather regular array of cells (the endothelial mosaic). Normally, all of the endothelial cells appear to be approximately the same size and shape [9,10]. In this study, corneal endothelial cells were evaluated

before and after LASIK combined with accelerated, high-fluence CXL.

Patients and methods

Study design

A prospective comparative nonrandomized interventional case series study was conducted in International Eye Center and Roaa Laser Vision Correction Center all through the period from March 2018 to January 2019. A total of 60 myopic eyes of 30 patients were distributed into two groups. Group A included 30 eyes of 15 patients, treated by LASIK, whereas group B included 30 eyes of 15 patients treated by LASIK associated with accelerated CXL.

The study was approved by the local research ethical committee of the Faculty of Medicine, Minia University, and was adherent to the tents of the Declaration of Helsinki. Detailed informed consent was taken from all patients for the surgical procedures and for inclusion in the study after thorough explanation of the procedures and all possible risks and benefits.

Inclusion criteria were patients more than or equal to 18 years old with myopia up to −8 D and astigmatism less than −5 D with pachymetry not less than 480 μm and the K-readings ranging from 41 to 48 D. Excluded criteria were patients with history of previous ocular surgery; systemic diseases that may affect corneal endothelium, for example, diabetes mellitus and autoimmune diseases; pregnancy; and lactation. Moreover, patients with KC, corneal opacities, or other ocular diseases were excluded.

All patients were subjected to complete preoperative assessment including complete ophthalmic and general history taking and complete preoperative ophthalmological examination including, slit-lamp examination of the anterior segment, manifest and cycloplegic refraction, measurement of the uncorrected and best-corrected visual acuity, measurement of the intraocular pressure, and fundus examination with examination of the peripheral retina. Rotating Scheimpflug camera examination was done using Oculus Pentacam (software version 6.02r10, Oculus Pentacam, Oculus Co., Irvine, California, United States) with assessment of preoperative pachymetry, horizontal and vertical corneal curvatures, and anterior and posterior corneal elevations in relation to the best-fit sphere (of 9-mm diameter). Specular microscopy was done using Tomey EM3000 (Nihon Optical Co., Ltd., Tomey Corporation, USA) for three times: first at baseline preoperatively and at 3 and 6 months postoperatively. Endothelium cell density (ECD), rate of polymegathism or coefficient of variation (CV), and rate of pleomorphism were evaluated.

Surgical procedure

The M-2 microkeratome (Moria, Antony, France) was used to create a 110 μm flap, and then the flap was reflected. Stromal ablation was done using the excimer laser (Visex Abbott Star S4 IR; visex: Abott Co. Santa, Ana, California, USA). The interface was irrigated using balanced salt solution. The flap was replaced and the interface was irrigated again, and the flap was repositioned according to the alignment marks. In group B, 0.1% riboflavin in 20% hydroxymethyl propyl cellulose solution (vibex rapid; Avedro Inc., Massachusetts, USA) was instilled every 30 s for 2 min to the stromal bed before the flap reposition, and then after flap reposition, the cornea was exposed to UV-A light of 366–374 nm at an irradiance of 30 mW/cm^2 for 3 min using OMNI, MMD (USA) machine.

Postoperative treatment included moxifloxacin hydrochloride 0.5% eye drops five times daily for 1 week, a combination of tobramycin and dexamethasone 0.1% eye drops four times daily and tapered over 3–4 weeks, and polyethylene glycol 400 mg five times daily for 1 month. Diclofenac sodium tablets were prescribed for pain as needed for first 2 days.

Postoperative evaluation was done after 1 day, 1 week, 2 weeks, 1 month, 3 months, and 6 months, with specular microscopy in the last two visits.

Statistical analysis

Statistical analysis was done using SPSS, version 19 (IBM Co., USA). Quantitative data were presented as mean and SD, whereas qualitative data were presented as frequency distribution. McNemar test was used to test the significant differences between preoperative values and 3-month postoperative values, preoperative values and 6-month postoperative values, and between 3-month postoperative values and 6-months postoperative value in each group. Comparison between groups was done by Mann–Whitney test. Spearman correlation was used. Probability of less than 0.05 was used as cutoff for significance.

Results

The study included 60 eyes of 30 patients allocated in two groups. Group A included 30 eyes of 15 patients

Table 1 Endothelial cell count (CD/mm^2) preoperatively and postoperatively in both groups

Endothelial cell count (CD/mm^2)	Group A *N*=30	Group B *N*=30	*P* value
Preoperatively			
Range	2582–2993	2427–3177	0.261
Mean±SD	2793.7 ±115.7	2745.8 ±199.7	
3 months postoperatively			
Range	2500–2890	1898–3122	0.040*
Mean±SD	2754.3 ±101.6	2647.1 ±257.3	
6 months postoperatively			
Range	2591–2975	1969–3240	0.081
Mean±SD	2782.7 ±107.2	2696.3 ±241.9	
P value			
Preoperative vs. 3 months	0.001*	0.005*	
Preoperative 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.

treated with LASIK, comprising four 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, comprising three males and 12 females. The mean age was 27.9±5.3 years. The mean ECD was 2793.7±115.7 CD/mm^2 in group A and 2745.8±199.7 CD/mm^{2}in group B. The changes in the mean ECD 3 and 6 months postoperatively in both groups are presented in Table 1. There was a statistically significant decrease in the mean ECD at 3 months postoperatively in both groups, with *P* value of 0.001 in group A and 0.005 in group B. The decrease becomes statistically insignificant by the sixth month postoperatively, with *P* values of 0.088 and 0.146, respectively. On comparing the two groups, a statistically significant difference was present only at third postoperative month (*P*=0.0040).

The mean polymegathism or CV was 37.3±3.9 in group A and 37.8±5.6 in group B. The changes in CV at 3 and 6 months postoperatively in both groups are presented in Table 2. In both groups, there was no statistically significant difference between preoperative and postoperative CV throughout the follow-up period. Moreover, on comparing both groups, there was no statistically significant difference at any time.

Table 2 Coefficient of variation preoperatively and postoperatively in the two groups

Coefficient of variation	Group A *N*=30	Group B *N*=30	*P* value
Preoperative			
Range	32–44	30–52	0.710
Mean±SD	37.3±3.9	37.8±5.6	
3 months postoperative			
Range	32–43	30–47	0.945
Mean±SD	37.9±2.7	38±4.6	
6 months postoperative			
Range	30–43	3–57	0.837
Mean±SD	36.8±3.2	36.4±8.3	
P value			
Preoperative vs. 3 months	0.318	0.782	
Preoperative vs. 6 months	0.354	0.366	
3 months vs. 6 months	0.027	0.232	

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.

Table 3 Mean percentage of hexagonal cells (pleomorphism) preoperatively and postoperatively in the two groups

Percentage of hexagonal cells (pleomorphism)	Group A *N*=30	Group B *N*=30	*P* value
Preoperative			
Range	37–62	31–60	0.458
Mean±SD	49.6 ±6.2	48.2 ±7.9	
3 months postoperative			
Range	34–60	31–58	0.256
Mean±SD	47±5.3	45.1 ±7.4	
6 months postoperative			
Range	41–59	33–61	0.733
Mean±SD	48.4 ±4.5	47.9 ±6.6	
P value			
Preoperative vs. 3 months	0.001*	<0.001*	
Preoperative vs. 6 months	0.177	0.782	
3 months vs. 6 months	0.043*	0.004*	

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 less than 0.05.

The mean percentage of hexagonal cells (pleomorphism) was 49.6±6.2 in group A and 48.2 ±7.9 in group B. The changes in pleomorphism at 3 and 6 months postoperatively in both groups are presented in Table 3. In group A, there was a statistically significantly decrease of pleomorphism at 3 months postoperatively compared with the preoperative value (*P*=0.001), whereas the decrease at 6 months postoperatively was statistically insignificantly (*P*=0.177). Similar results were obtained in group B, with *P* values of less than 0.001 and 0.782 at 3 and 6 months after surgery,

respectively. On comparing both groups, there was no statistically significant difference in pleomorphism at all times.

The mean maximum endothelial cell size was 938.6 ±233 μm in group A and 971.6±268.8 μm in group B. In group A, there was a statistically insignificant decrease to 900.3±169.7 μm at 3 months postoperatively (P=0.121) and to 903.1±183.2 μm at the end of follow-up at 6 months (P=0.184). Moreover, in group B, the mean maximum endothelial cell size decreased insignificantly to 946 ±237.5 μm at 3 months (P=0.587) and to 866.2 ±247.1 μm at 6 months postoperatively (P=0.090). There were no statistically significant changes comparing the two group preoperatively (P=0.613) and at 3 months (P=0.394) and 6 months (P=0.515) postoperatively.

On the contrary, the mean minimum endothelial cell size decreased significantly in group A, from 95.5±18.2 to 87.7±13.6 μm at 3 months (P=0.002) and to 87.8 ±10.9 μm at 6 months postoperatively (P=0.009). However, the change in the mean minimum endothelial cell size was statistically insignificant in group B from 95.3±19.4 to 98.4±17.8 μm at 3 months (P=0.354) and to 95.6±19.4 μm at 6 months postoperatively (P=0.939). There was a statistically significant difference on comparing the two group at 3 months (P=0.011) and statistically insignificant changes at 6 months postoperatively (P=0.061).

Discussion

Concurrent use of accelerated CXL with LASIK can decrease the risk of postoperative corneal ectasia and regression of correction with time, especially in young patients and those with high myopia [11,12]. A significant limitation of accelerated CXL is its effect on corneal endothelium [13,14]. In the standard CXL protocol, UV-A energy of 3 mW/cm^2 is used for 30 min in conjunction with the application of hydrophilic riboflavin 0.1%. This causes a significant decrease in UV-A light energy reaching corneal endothelium by up to 95% (only 0.15 mW/cm^2 with corneal thickness of >500 μm) [15]. To reduce the time of the procedure, according to the Bunsen–Roscoe law of reciprocity, a higher intensity of the UV-A energy is required [16]. Therefore, treatment using UV-A energy of 3 mW/cm^2 for 30 min is equivalent to the use of 9 mW/cm^2 for 10 min or 30 mW/cm^2 for 3 min [17]. The higher UV-A intensity radiation may lead to the damage of the nerve plexus and impair the endothelial pump performance. The sub-basal nerve plexus produces transported neuropeptides including calcitonin-gene-related peptide and substance P. They have a role in assisting the transmission of signals through the Na/K-ATPase pumps in corneal endothelium [18].

The current study compared postoperative endothelial cells parameters with the preoperative values both in eyes subjected to LASIK alone and those subjected to LASIK combined with accelerated cross-linking. Regarding the mean ECD, no significant change was present by the end of the 6-month follow-up period (P=0.146). Moreover, on comparing both groups with each other, there was an insignificant difference (P=0.081). This indicates that combined surgery has no significant effect on postoperative mean ECD. These results are consistent with other studies that evaluated corneal endothelium after refractive surgery or CXL and found little or no change in ECD [4,19]. Moreover, Wu *et al.* [20] had insignificant endothelial cell changes in both density (ECD) and morphology (CV and percentage of hexagonal cells following accelerated CXL 30 mW/cm^2 for 90 s. Similarly, in the current study, on comparing CV and percentage of hexagonal between the two groups, there were no significant changes preoperatively and after 3 months and 6 months following the procedure. Moreover, in each group, no significant difference was found between preoperative and postoperative CV and pleomorphism.

Moreover, our results in evaluating cells (pleomorphism) preoperatively and postoperatively (3- and 6-month follow-up) and in comparing between the two groups, there is no significant difference preoperatively (P=0.458) and postoperatively at 3 months (P=0.256) and 6 months (P=0.733).

On the contrary, in a study that used CXL in the treatment of KC and post-LASIK ectasia, found significant endothelial cell changes in both ECD and morphology (CV and percentage of hexagonal cells) following accelerated CXL (18 mW/cm^2 for 5 min). These changes were observed at the first week and the first month. Then, corneal endothelial count returned to the baseline values at 6 months, whereas the percentage of hexagonal cells and CV returned to their base values only at 3 months [21]. In the study of Badawy [20], UV-A irradiance of 30 mW/cm^2 was used for 3 min. The changes did not return to their basal values. The statistically significant changes in ECD and CV were persistent

until the end of 1-year follow-up [22]. In contrast, Cınar *et al.* concluded that accelerated CXL had negligible effects on ECD at 6-month follow-up of 23 patients with progressive KC treated by accelerated CXL (9 mW/cm^2 for 10 min). The endothelial cell changes were statistically insignificant (P=0.082) [23]. In another comparative study by Kanellopoulos between standard CXL (3 mW/cm^2 for 30 min) in one eye of 21 patients and accelerated CXL (7 mW/cm^2 for 15 min) in the fellow eye, both accelerated and standard CXL had similar safety profile on the corneal endothelium [24].

Conclusion

LASIK with accelerated CXL is safe and has no significant adverse effect on corneal endothelium. The low-risk profile together with the significant improvement in refractive stability potentiate the use of LASIK-CXL as a promising adjunct to LASIK in decreasing the possibility of enhancement procedures, especially in eyes with high errors.

Limitations of the study include the short-term follow-up period and the relatively small sample size.

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Conflicts of interest

There are no conflicts of interest.

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