Original Article

Effect of Mitomycin-C on Endothelial Cell Density andPolymegathism in Laser Assisted Sub-Epithelial Keratectomy

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Abstract

Purpose: To evaluate the corneal endothelial cell density and polymegathism after laser assisted keratomileusis with mitomycin C.

Patients and Methods: One hundred and three eyes from 52 patients undergoing laser assisted sub-epithelial keratomileusis using mitomycin-C 0.02 % for 30 seconds entered this study. Specular microscopy was utilized to study the corneal endothelial cells before and one year after the surgery to evaluate the endothelial cell density and polymegathism. The corneal thickness was measured before and one year after the surgery.

Results: The mean endothelial cell density before surgery was 2870 ± 368 /mm^2, which changed to 2828 ± 300 /mm^2 one year after the surgery (P = 0.182). Endothelial cell size variation coefficient was 24 ± 7 before the surgery, which changed to 25 ± 7 one year after the surgery (P = 0.039). The average stromal ablation dept was 65 micron (22 to 131 micron) and the remaining corneal thickness one year after the surgery was 488 ± 42 micron (405 to 567 micron) (P < 0.001).

Conclusion: Using mitomycin C (0, 02 % for 30 seconds) during the laser assisted sub-epithelial keratomileusis did not significantly change the corneal endothelial cell density one year after the surgery but the change in polymegathism was significant.

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Introduction

Laser-Assisted in Situ Keratomileusis (LASIK) is a widespread surgical procedure because of its advantages such as, less pain, rapid visual rehabilitation and lower incidence of complications [1]. A modification of this method called Laser-Assisted Subepithelial Keratectomy (LASEK) was first introduced by Massimo Camellin [2]. This surgical procedure is used to treat nearsightedness, farsightedness and astigmatism and is also an effective technique to correct refractive errors in eyes with high myopia, thin corneas and damaged retina. It reduces some complications of LASIK surgery like corneal ectasia [3]. At the end of this procedure, the corneal epithelium is covered again by the epithelium. This leads to better wound healing, protects the bare stroma from tear inflammatory cells and reduces the corneal haze and postoperative pain which helps to maintain the stability of the cornea [3]. In addition, because there is no incision in the cornea it maintains the corneal stability like photorefractive keratectomy (PRK) and by eliminating the flap complications of LASIK it will be possible to treat patients with thin corneas, as well as patients with large pupils [3-5].

In LASEK the surgeon tries to keep the corneal epithelium to reduce the corneal opacity (haze), which is a major complication after application of PRK, but corneal opacity is still a main complication of this procedure [2,3].

In LASEK and PRK, mitomycin-C is used as a topical treatment to help preventing the corneal opacity [6-9]. Animal models have shown that mitomycin-C can cause suppression of the activity of keratocytes and fibroblasts and prolong keratocytes apoptotic process after LASEK and PRK which can explain its effect on corneal opacity [9-17]. Although many clinical studies have indicated the safety of the use of mitomycin-C after LASEK and PRK surgery [18-21], however the issue of toxicity of mitomycin-C on endothelial cells remains controversial. Some studies, have indicated significant changes in corneal endothelial cells after application of mitomycin-C [22,23]. They have also indicated that the toxicity of mitomycin-C on endothelial cells depends on the length of time or the concentrations of the drug [24].

Since there are different reports considering the rate of change of endothelial cells after photorefractive surgery with mitomycin-C, this study was performed to evaluate the effects of mitomycin-C on endothelial cell density and coefficient of variation of cell size one year after LASEK surgery with mitomycin-C application.

Patients and Methods

Patients

In a retrospective study, 103 eyes of 52 patients who underwent LASEK surgery for myopia and myopic astigmatism correction with mitomycin-C were evaluated. All patients were from Negah Eye hospital, Tehran, Iran. Patients did not wear soft contact lenses for one week or hard contact lens or 6 to 8 weeks before the surgery. Patients with any systemic disease or a history of systemic or topical medications with an impact on the endothelium of the cornea were excluded from the study.

Preoperative examination

Preoperative examination included the evaluation of uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) using Snellen chart (Auto chart projector Nidek CP 670), manifest and cycloplegic refraction, slit lamp examination, measurement of eye pressure using tonometry (Goldmann applanation), and dilated

fundus examination. In all patients, corneal topography was performed to evaluate the thickness and curvature of the cornea using Orbscan II (Bausch & Lomb's Orbscan IIz) to ensure there is no indication of keratoconus. Study on endothelial cells was performed using a non-contact specular microscopy system (Topcon SP2000P, Tokyo, Japan).

Postoperative visits

Eyes were evaluated one to four days after the surgery, in the third and eighth weeks, as well as six and twelve months after the surgery. Postoperative examination included UCVA and BCVA measurements, refraction measurement, examination of the anterior segment using slit lamp, measurement of the corneal thickness using Orbscan II and measurement of the eye pressure. Calculation of the corneal endothelial cell density and coefficient of variation of endothelial cells using non-contact specular microscopy was performed one year after the LASEK surgery.

Assessment of endothelial cells

To evaluate changes in endothelial cells all patients were examined before and one year after LASEK procedure. Corneal endothelial cell density and size variation coefficient was calculated before and one year after the surgery by a qualified optometrist and using non-contact specular microscopy (Topcon SP2000P, Tokyo, Japan). This device uses frame method, to automatically select 20 endothelial cells and provides an estimate for the total endothelial density. The endothelial cell density, the coefficient of variation of corneal endothelial cells, the average area of endothelial cells, and central corneal thickness was investigated. During the specular microscopy patients were asked to look directly towards the central objective to ensure that the center of the cornea is examined.

LASEK surgery technique

After dropping a few drops of tetracaine 5.0 percent (Sina Darou, Tehran, Iran), 20 % alcohol diluted with BSS solution was applied for 20 seconds in a standard 8 mm corneal marker to the center of the cornea. The eye was rinsed with BSS solution and the corneal epithelium was removed using a hockey knife. The corneal ablation was performed using a Technolas machine (Technolas 217z100, Bausch & Lomb Surgical Rochester, NY) with front wave method by two experienced surgeons. For all patients, a sponge soaked in Mitomycin-C 0.02 %, was applied for 30 seconds in the laser area. Using the sponge prevents the release of the drug into the surrounding tissue. At the end of the procedure the eye and flap were rinsed again with 50 ml of cold BSS and the flap was returned slowly to its location. A bandage contact lens (Ciba Vision, Night and Day ®) was placed and a drop of chloramphenicol and a drop of betamethasone was applied to end the procedure. From day one after surgery chloramphenicol drops were used every 6 hours for one week. Also fluorometholone (FML) drop 1 percent was applied every 6 hours for one month, every 8 hours in the second month, and every 12 hours in the third month. Diclofenac was applied every 12 hours in the first three days if the patient complained of pain. Contact lens bandage was removed after complete epithelium recovery.

Statistical Analysis

To describe the data, average (median and interquartile range), standard deviation, frequency and percentage were used. for comparison of endothelial cells before and after the intervention paired T-test

was used. SPSS software (Version 20. Armonk, NY: IBM Corp.) was used for statistical analysis. P values less than 0.05 were considered statistically significant.

Results

One hundred and three eyes from 52 patients who underwent LASEK using mitomycin-C for were included in the study. The average age of patients was 27 years with a range of 19 to 48 years, with 17 patients (69/32 %) being male and 35 patients (30/67 %) being female. The degree of myopia, astigmatism, spherical equivalent, corneal thickness and corneal thickness before and after surgery is displayed in table1.

Table 1: Demographic findings among patients entering the study.

Variable	Minimum	Maximum	Mean
Age	19	48	27.06
Sphere (Diopter)	-8.75	0.25	-3.41
Cylinder (Diopter)	-3.75	0.00	-0.94
Spherical Equivalent (Diopter)	-9.00	-0.68	-3.88
Ablation Dept (Micron)	22	131	65
Preoperative Corneal Thickness (Micron)	473	616	547
Postoperative Corneal Thickness (Micron)	405	567	488

correction of myopia and myopic astigmatism

The average central corneal thickness was 547 ± 37 micrometers before the surgery and 482 ± 42 micrometers after the surgery (P = 0.001) (Table 2). The average corneal endothelial cell density was 2870 ± 368 before the surgery, and 2828 ± 300 one year after surgery (P = 0.182) (Table 2). Preoperative corneal endothelial cell size variation coefficient was 24 ± 7 percent before the surgery, and 25 ± 7 percent one year after the surgery (P = 0.039) (Table 2).

Table 2: Comparison of corneal thickness and corneal endothelial cell parameters bebore and after LASIK surgery.

Variable	Pre Surgery	Post Surgery	P
Central Corneal Thickness	547 ± 32	488 ± 42	< 0.001
Mean Corneal Endothelial Cell Size (Square micrometer)	354 ± 47	358 ± 39	0.432
Corneal Endothelial Cell Density	2870 ± 368	2828 ± 300	0.182
Corneal Endothelial Cell Size Variation Coefficient (%)	24 ± 7	25 ± 7	0.039

Furthermore, the mean preoperative endothelial cell area was 354 ± 47 square micrometers before the surgery and 358 ± 39 square micrometers after the surgery (P = 0.432) (Table 2). No intraoperative complications such as defects in the repair of corneal epithelium or keratitis after the surgery was observed. Also no significant corneal opacity was observed in any of the patients.

Discussion

It has been reported that intraoperative use of direct mitomycin-C in LASEK and PRK procedures remarkably reduces the corneal opacity [9,13,14,25]. Concerns about the complications after mitomycin-C usage in treatment of pterygium [26,27], glaucoma [28] and ocular surface neoplasia [29] have been raised. Although these complications have not been reported after its usage in LASEK and PRK procedures [30], however, studying the subclinical toxic effects of mitomycin-C on cornea is needed. Mitomycin-C has long-term effects on cells that have a high proliferation rate like keratocytes and corneal epithelial cells, however, there is also a possibility of damage to nonproliferative cells like endothelial cells [20,30]. In studies on rabbits it has been demonstrated that mitomycin-C causes corneal edema and

endothelial cell apoptosis which is related to its concentration and corneal contact time [30]. The effects of mitomycin-C on human cornea may be different because rabbit corneal endothelial cells have a high degree of proliferation compared to human corneal endothelial cells.

There is no report of human corneal edema after corneal refractive surgery using mitomycin-C (0.02 %), so the possibility of severe damage to the endothelial cells is unlikely [30].

In our study, application of mitomycin-C (0.02 %) caused no significant change in the number of endothelial cells one year after LASEK surgery but other studies have reported conflicting results. Lee et al., did not find a statistically significant change in the number of endothelial cells 3, 6 and 16 months after PRK surgery using mitomycin-C (0.02 %) for 30 seconds [31]. Also Goldsberry et al., [21] did not find a significant change in the number and morphology of corneal endothelial cells 12 months after PRK surgery with mitomycin-C applied for 12 seconds. Diakonis et al., [32] in a prospective randomized study compared 15 eyes undergoing PRK with the use of mitomycin-C (0.02 %) for 15 seconds with opposite eyes from same patients undergoing Epi-LASIK without the use of mitomycin-C, 1 and 3 months after the surgery. No significant difference in the number of endothelial cells was observed between the two groups. The results of these studies are all consistent with our findings. In contrast, Morales et al., [22] in a prospective study performed on 18 eyes undergoing PRK found a significant reduction in corneal endothelial cells three months after mitomycin-C (0.02 %) usage for 30 seconds. Also Nassiri et al., [23] in a non-randomized prospective study on 162 eye undergoing PRKI found a significant reduction in the number endothelial of cells, six months after the surgery with mitomycin-C applied for 10 to 50 seconds. This reduction was directly related to the duration of mitomycin-C contact with the corneal surface.

The duration of mitomycin-C usage in our study was 30 seconds and its concentration was (0.02 %) which indicated no significant change in the number of endothelial cells. This duration and concentration was similar to Zhao et al., [20] who used mitomycin-C (0.02 %) for 15 seconds and Goldsberry et al., [21] who used it for 12 seconds. Most authors recommend a duration of 15 to 30 seconds for mitomycin application during LASEK and PRK surgeries [20,21].

The endothelial cell size variation coefficient showed a significant change in our study one year after the LASEK surgery using mitomycin-C. This finding is contrary to the results of Goldsberry etal., [21] who have reported no significant change 12 months after PRK surgery using mitomycin-C for 12 seconds. Also Zhao et al., [20] found no significant change in endothelial cell size variation coefficient after LASEK surgery using mitomycin-C for 15 seconds. Although the lack of a control group was a limitation of our study, the relatively high number of participants and its one year followup should be considered as its advantages. In opinion of the authors the difference in results of studies on the effect of mitomycin-C on corneal endothelial cells might be due the multiplicity of variables affecting these cells, including the duration of mitomycin-C application, the remaining stroma of the cornea and the difference in how the specular microscopy is performed. So in order to achieve more reliable results prospective studies with a control group and a standard time for application of mitomycin-C is recommended.

Conclusion

Using mitomycin - C (0, 02 % for 30 seconds) during the laser assisted sub-epithelial keratomileusis did not significantly change the corneal endothelial cell density one year after the surgery but the change in polymegathism was significant.

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Footnotes and Financial Disclosures

Conflict of Interest:

The authors declare no conflict of interest with the subject matter of the present manuscript.