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THE EFFECT OF MITOMYCIN-C ON CORNEAL ENDOTHELIAL CELL COUNT AFTER PHOTOREFRACTIVE KERATECTOMY

[2]Giyath Aldeen Thajeel Neamah, MBChB, FIBMS;
[1]Hashim Al Ameedee, MBChB, CABO;
[3]Ahmed M. Al Samak, MBChB, CABO;
[1]Alyaa Abood Kareem, MBChB, FIBMS.
Author Affiliations: [1]Department of Ophthalmology, Kufa University, Najaf, Iraq; [2]Department of Ophthalmology, Jabir Ibn Hayyan Medical University, Najaf, Iraq and [3]Department of Ophthalmology, Basrah University, Basrah, Iraq.

Abstract
Purpose: The goal of the study is to assess changes in central corneal endothelial cell density after photorefractive keratectomy (PRK) with mitomycin-C (MMC) 0.02% in patients with myopia.
Methods: This case control study included 66 eyes of 33 patients (22 cases&11 controls), aged 18 to 40 years. All were myopic (spherical equivalent refractive error -3.00 to -10.00D), who had photorefractive keratectomy (PRK). The treatment group was exposed to single intraoperative application of MMC 0.02% for 50 seconds while; the control group had the surgery without the use of MMC. Specular microscopic assessment was done preoperatively and repeated 2 weeks, 1 month and 3 months after surgery for each eye to set changes in central corneal endothelial cell density (ECD) and coefficient of variation in cell size (CV).
Results: Forty-four eyes of 22 cases with mean age of 27.5±6.16, range 18 to 40 years) and twenty-two eyes of 11 control patients with mean age of 25.4±5 years (range 19 to 34 years) underwent photorefractive keratectomy. Mean preoperative spherical equivalent refractive error was −6.2±1 (range −3.00 to −10.00). Three months after the operation the mean ECD was reduced significantly by 6.8% ($p<0.05$) from 2702.09 cells/mm² preoperatively to 2517.41cells/mm² for right eye and 5.4%($p<0.05$) from 2687 cells/mm² to 2543 cells/mm² for the left eye in the treatment group whereas, the control group showed slight reduction in ECD (3%) from 2713 cells/mm² to 2630 cells/mm² ($p= 0.32$). There was no significant change in coefficient of variation in cell size (CV), ($P=0.52$) at 3 months.
Conclusion: Application of MMC 0.02% for 50 seconds during PRK in patients with myopia significantly affects central corneal endothelial cell density after three months follow up period.
Key words: photorefractive keratectomy, endothelial cell, Mitomycin-C, Myopia

Introduction:
Mitomycin-C stop cells from proliferation by cross-linking DNA [1]. It has been widely used intraoperatively for pterygium excision, trabeculectomy, and surface ablation procedures. In addition, it is used topically in a cyclic fashion for primary or recurrent ocular surface squamous neoplasia [2]. It has been found safe and effective in animals, MMC was suggested for application during surface ablation procedures to decrease postoperative haze [3]. It can effectively reduce haze and improve the predictability of visual outcomes following refractive surgery. Despite these advantages, MMC can potentially damage all three main corneal cell types including epithelial (differentiated epithelium and limbal cells), stromal (keratocytes), and endothelial cells.

In the post-PRK cornea, it blocks the proliferation of activated keratocytes and other progenitor cells of myofibroblasts, which in turn prevents scarring [4]. The percentage of corneal haze will be higher as the depth of the ablation is increased. Consequently, patients with medium to high myopia (greater than six diopters) will have a higher risk of a haze than those who are lesser degree of myopia [5]. Moreover, Tabbara, et al found an elevated risk of corneal haze following PRK in Saudi patients with brown irides when compared to Caucasian patients with blue irides [6]. High ultraviolet light exposure may serve as an additional risk factor for later occurring haze. Consequently, many surgeons recommend using UV light protective sunglasses, especially in the first year following surgery.

Endothelial cell dysfunction and loss-through surgical injury, inflammation, or inherited disease (eg, Fuchs endothelial dystrophy) may cause endothelial decompensation, stromal odema and vision failure. To date, there has been conflicting evidence about whether mitomycin-C use results in a decrease in the number of endothelial cells in treated eyes, with some studies demonstrating a decline and others noting no statistically significant difference in cell counts [7].

The popularity of PRK decreased in the late 1990s when LASIK began to be performed because of LASIK's faster recovery of vision and decreased postoperative discomfort. Although more LASIK than surface ablation procedures are still performed, the number of surface ablations has been increased in recent years [7]. PRK remains an alternative for certain indications, including irregular or thin corneas, epithelial basement membrane disease, previous corneal surgery, such as penetrating keratoplasty and radial keratotomy and treatment of some LASIK flap complications such as incomplete or buttonholed flaps [8].

The incidence of this complication is much lower today than in the mid-1990s due to surgeons' better understanding of the chief risk factors for haze [9,10]. Manufacturers have also recognized that they can design excimer lasers to reduce the risk of haze by creating a smoother ablation profile and minimizing the generation of heat [8]. In fact, although the technology has improved and some of the risk factors are now well known, haze remains the major complication after PRK. other complications of PRK includes, persistent epithelial defect,sterile infiltrate, infectious keratitis and central toxic keratopathy.

Corneal haze, reduced markedly with the use of adjunctive mitomycin-C; subsequently, the use of PRK for higher levels of myopia has increased [11]. A proper dilution of the drug is crucial with the standard concentration of topical MMC (0.02%) being more effective than a lower dose (0.002%) in preventing postoperative haze following surface ablation for high myopia, without inducing any serious complications [11,12]. The duration of MMC exposure may be risk factor for toxicity [13].

Since MMC is potentially toxic, the goal is to use the smallest dose that will reliably prevent haze. Dosing can be adjusted by altering either the concentration of drug applied or the length of time it is left on the eye Both approaches have been tried [14].

Patients and methods:
This case control study included 66 eyes of 33 patients (22 cases &11 controls), female subjects(15 cases&6 controls),aged 18 to 40 years all of them are myopic (spherical equivalent refractive error -3.00 to -10.00D). Informed consent has been obtained from all patients.

The following cases, where refractive surgery is contraindications, have been excluded :keratoconus, corneal dystrophy or degeneration, glaucoma ,history of ocular trauma or surgery. Informed consent was obtained from all participants after explaining the purpose of the study.

Preoperative evaluation include history taking, measurement of uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA) using the Snellen chart, manifest and cycloplegic refraction, slit lamp examination of the anterior segment& dilated fundus examination, measurment of intraocular pressure , measurment of central corneal pachymetry using an ultrasonic contact probe (A/B scan; Sonomed Inc., Lake Success, NY, USA), corneal topography, Specular microscopy (EM 3000, TOMY., Tokyo, Japan) to determine central corneal endothelial cell density (ECD), mean cell area (MCA), and coefficient of variation in cell size (CV). To reduce sampling error, the clearest specular image containing at least 100 endothelial cells in the center of the field was used. Examination was performed by the same operater.

***Surgical technique**:*
All participants in the cases group underwent photorefractive keratectomy by a single ophthalmologist (at AL Hakeem hospital, Laser Center in Ophthalmology Department) using a scanning slit excimer laser machine (Nidek, USA), with an emission wavelength of 193 nm, fixed pulse repetition rate of 50 Hz, and radiant exposure of 400 mJ.

The control group underwent the surgery at AL Basrah teaching hospital without the use of MMC intraoperativly. Topical tetracaine hydrochloride 0.5% eye drops were used to anesthetize the cornea. Antisepsis was performed by applying 10% povidone-iodine solution to the skin of the eyelids and periocular area for 1 minute and the eyes were washed out by 20 mL of balanced salt solution (BSS).

The epithelium was mechanically removed in the 8.0 mm central cornea. Thereafter, ablation was performed using the Planoscan software. Optical zone was 6.0 mm and manifest refractive error was considered as the target for correction in all cases. Subsequently, a sponge, 7.0 mm in diameter, soaked with mitomycin-C 0.02% (0.2 mg/mL, diluted in BSS), was applied over the ablated surface for 50 seconds in the cases group. This was followed by copious irrigation with BSS, and fitting a contact lens at the conclusion of the operation.

Follow-up:
Postoperatively, all participants were given steroid/antibiotics eye drops (ofloxacillin+dexamethasone) every 4 hours, with NSAID eye drop (Acular) on need for 5 days. Once re-epithelialized (usually between 3 to 5 days), the contact lens was removed. Thereafter they were given dexamethasone eye drop 0.1% 6 hourly for 2 weeks then fluoromethalone eye drop 0.1% 6 hourly for 3 weeks.

Follow-up examinations were scheduled 1, 5, 14, 30 days and 3 months postoperatively. At each examination except on days 1 UCVA, BCVA, manifest refraction, and tonometry were checked. Specular microscopy was repeated 2 weeks,1 month and 3 monthes after surgery..

The same steps were done for the control group but without the use of mitomycin-C intraoperativly. postoperatively the patients was given the same medications and follow up examinations were scheduled 1,5,14 and 30 days. Specular microscopy was repeated 1 month after surgery.

Statistical Analysis:
Data were presented as mean ± standard deviation. SPSS (statistical package for social sciences) version 20 in which paired t-test and independent sample t-test were used to compare pre and postoperative ECD and CV. P-values less than 0.05 were considered as statistically significant.

Results:
Forty-four eyes of 22 cases with mean age of 27.5±6.16 years (range 18 to 40 years) and twenty-two eyes of 11 controls patients with mean age of 25.4±5 years (range 19 to 34 years), with no significant difference between male and female distribution (p =0.652) underwent photorefractive keratectomy (Table 1). Mean preoperative spherical equivalent refractive error was −6.2±1. (range, −3.00 to −10.00 D) which was reduced to −0.4±0.5 postoperatively (P<0.001).

Three months after the operation the mean ECD was reduced by 6.8% (p<0.05) from 2702.09 cells/mm² preoperatively to 2517.41cells/mm² for right eye and 5.4%(p<0.05) from 2687cells/mm² to 2543 cells/mm² for the left eye. There was no significant change in coefficient of variation in cell size(CV) mean 44.64 preoperatively versus 40.77, 3 months postop(P=0.52) (Table 2). ECD doesn't change significantly in the control group one month after the operation (from 2713 cells/mm² to 2630 cells/mm²).

There is no significant change in CV in the control group mean 42.10 preoperatively versus 41.30 one month postoperative. Neither the ECD nor the CV in cell size differ significantly between the case and control groups one month after the operation (Table 2).

None of the patients developed postoperative complications such as persistent epithelial defect, infectious keratitis, or significant haze formation.

Discussion:
Mitomycin-C is used commonly after photorefractive keratectomy to modulate the corneal wound healing response and prevent occurrence of primary and recurrence of preexisting haze formation [15,16].

The original procedure of intraoperative MMC exposure suggested a twelve seconds to Two minutes time interval. Because of concerns regarding MMCs safety, a series of alterations on the usage of MMC have been suggested. This has led to decrease exposure time of MMC with the aim of achieving an equivalent effect on haze inhibition with less potential toxic effect.

If the concentration is too high, MMC may be toxic, producing opacity and corneal melting [9]. Administration of MMC 0.02% applied for 12 seconds following PRK did not have a significant effect on quantitative endothelial cell density or qualitative morphometric parameters [10]. To the contrary, in a non-randomized controlled trial, Nassiri et al. noted that the prophylactic use of diluted intraoperative MMC 0.02% solution caused corneal endothelial cell loss [8].

Several studies have investigated the effect of a single intraoperative dose of MMC during refractive surgery on the corneal endothelium. Some clinical [8,17] and laboratory studies have reported significant corneal endothelial toxicity [18-20]. However, the majority of clinical studies have reported no significant change in corneal endothelial density or morphology with follow-up period ranging from 3 to 18 months [10, 12, 15, 21-23]. Most studies on MMC have employed a short duration of exposure, less than 20 seconds. Since different

durations of MMC application have been used, discrepancies in the findings of these studies make it difficult to reach a definite conclusion on the safe concentration of MMC [15].

In the current study, reduction in cell density was 6.8% in the RE and 5.4% in the LE while the control eyes show reduction rate 1.05% in the RE and 3% in the LE. There was no significant change in the morphological features of the endothelial cells including coefficient of variation in cell size. The results of this study are supported by previous two studies. A prospective, randomized study from Mexico that used the fellow eye as a control and used 0.02% mitomycin-C for 30 seconds found that the endothelial cell loss was statistically significant in the mitomycin-C group at one month (14.7%) and three months (18.2%) postoperatively, while corresponding figures were not significantly changed in the BSS group (4.3% and 5.0% respectively) [17].

Another study divided 81 patients with low to moderate myopia (162 eyes) into three groups: bilateral mitomycin-C application; unilateral and untreated. Overall, 76 eyes were treated with MMC. Six months postop, the researchers found a decrease in endothelial cell density (-14.8%t): that was statistically significant compared to control eyes (-5.1%). The MMC protocol consisted of a cellulose sponge pledget soaked with MMC 0.02% being placed on eyes with ablation depths of 75 µm or deeper. The duration of contact ranged from 10 to 50 seconds, with deeper ablations receiving a longer contact time. The investigators report that longer drug contact time and male sex were associated with greater cell loss [8].

In contrast, there have also been a number of studies that have not found a connection between MMC and endothelial cell loss. A recent study showed that the application of MMC 0.02% for 40 seconds during PRK in patients with moderate myopia did not significantly affect central corneal endothelial cell density and morphology after a 6 month follow up period [21].

Goldsberry and his associates published a study in 2007, in which 16 eyes with a planned ablation depth greater than 75 µm underwent PRK followed by 0.02% MMC application for 12 seconds. They examined the endothelial cells preoperatively and at least a year postoperatively with specular microscopy. They didn't find any significant differences in cell density or the percentage of hexagonal cells present [10].

The decrease in keratocyte density after MMC application is correlated with its concentration and to a lesser extent exposure time. A similar association can be expected for the effect of MMC on endothelial cells. Theoretically, another determinant could be the depth of ablation; deeper ablation leaves a thinner residual stroma, allowing the drug to penetrate and concentrate in the anterior chamber to a higher concentration. Increasing MMC exposure time to match the greater depth in the higher myope is probably not advisable. The increased ablation depth for high myopia may be associated with deeper penetration of mitomycin, which could reach levels very close to the endothelium. There is no actual reason to use a long exposure time for haze prevention. A “standard” 12 seconds of the exposure time has been found to work perfectly well in reducing postoperative haze without compromising endothelial cell count.

Regarding the efficacy of using an even lower dose of mitomycin-C 0.002% vs. 0.02%, Krueger et al. has studied this in detail, and found significantly less haze with the standard-dose group in higher myopia (>-6 D) and higher ablation depth (>75 µm) at all postop time points compared to the lower dose. Overall, it appears the standard dose is probably preferable.

Our study showed that application of MMC 0.02% for 50 seconds during photorefractive keratectomy in patients with myopia significantly reduced central corneal endothelial cell density after a 3 months follow up period (reduction rate was 6.8%).

Although mitomycin-C is important as adjunctive agent for ocular surgery and seems to be effective in prevention of corneal haze after PRK, its use endangers endothelial cell count. The following recommendation should be considered:

1. The use of MMC should be avoided in cases suspected to have low endothelial cell counts such as older age patients, previous corneal trauma or surgeries, patient with chronic uveitis and corneal endothelial dystrophies.
2. The duration of application of MMC during PRK should be reduced if appropriate especially for low degree of refractive error.
3.Measurment of endothelial cell counts should be done in all suspected cases

Compliance with Ethical Standards:

Conflict of Interest:

Hashim Al Ameedee declares that he has no conflict of interest.
Giyath Aldeen Thajeel Neamah declares that he has no conflict of interest.
Ahmed M. Al Samak declares that he has no conflict of interest.
Alyaa Abood Kareem declares that she has no conflict of interest.

Ethical approval: All procedures performed in this study were in accordance with the ethical standards of the local research committee and with the 1964 Helsinki declaration and its later amendments.
Informed consent: Informed consent was obtained from all individual participants included in the study.

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