

## Mitomycin-C Application Before versus After Scleral Flap Dissection in Trabeculectomy; a Randomized Clinical Trial

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### Abstract

**Purpose:** To compare trabeculectomy with mitomycin-C (MMC) application before versus after scleral flap dissection in terms of corneal endothelial cell loss and surgical outcomes.

**Methods:** In this double blind clinical trial, patients were randomized to MMC 0.02% application before (group A) or after (group B) scleral flap dissection. The main outcome measure was corneal endothelial cell density; secondary outcome measures included IOP, glaucoma medications, success rates (IOP  $\leq 21$  and  $\leq 18$  mmHg, defined as criterion 1 and 2, respectively) and complications.

**Results:** Overall, 99 eyes of 99 subjects including 72 male and 27 female subjects were operated and followed for at least 6 months. The study groups were comparable in terms of baseline variables. Outcomes of surgery were similar at six months in terms of IOP ( $11.8 \pm 5.8$  vs.  $11.7 \pm 5.5$  mmHg,  $P=0.88$ ) and number of medications ( $0.2 \pm 0.6$  vs  $0.1 \pm 0.4$ ,  $P=0.45$ ). Overall success was comparable at months 1 and 3, but higher in group B at month 6 (82.0% vs. 63.3%,  $P=0.036$  for criterion 1 and 78.9% vs. 59.2%,  $P=0.044$  for criterion 2). Hypotony was more prevalent in group B (8.0% versus 2.0%) but the difference was not significant ( $P=0.38$ ). Endothelial cell density loss ( $2.2 \pm 7.3$  vs  $0.9 \pm 6.3\%$ ,  $P=0.567$ ) was comparable between the study groups.

**Conclusion:** Corneal endothelial loss following trabeculectomy was comparable with MMC application before and after scleral flap dissection. The sequences were comparable in terms of postoperative IOP and glaucoma medications. Overall success rate was higher at six months in group B and the rate of hypotony was also higher, although insignificantly.

**Keywords:** Corneal Endothelial Cell Density; Mitomycin-C; Success Rate; Trabeculectomy

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### INTRODUCTION

The most common cause of failure of glaucoma filtering procedures is scar formation at the site of surgery.<sup>[1]</sup> Mitomycin-C (MMC) is a potent anti-fibrotic agent which has been widely used intraoperatively to improve the outcomes of surgery. Mitomycin C is an antibiotic agent with antiproliferative properties and is metabolically activated into an alkylating agent which cross-links DNA, acting as a cell cycle nonspecific cytotoxic agent. It not only inhibits DNA replication,

but also hinders mitosis and protein synthesis. Mitomycin C does not interfere with a metabolite required for DNA, RNA, or protein synthesis and therefore is not classified as an antimetabolite. MMC has an inhibitory effect on fibrosis and vascular growth both of which play important roles in tissue healing and scar formation.<sup>[2]</sup>

The results of glaucoma surgical procedures including trabeculectomy have been improved and

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lower intraocular pressure (IOP) levels have been achieved since the introduction of MMC. This improved success however, has been associated with a higher risk of both early postoperative complications including shallow anterior chambers, transient hypotony, wound leaks, conjunctival necrosis and choroidal effusions, and late complications such as hypotony maculopathy, excessively thin walled blebs, late onset bleb leaks and bleb associated infections.<sup>[3,4]</sup> The ideal concentration and duration of MMC application for trabeculectomy has not been established and surgeons may vary in their choice of concentration from 0.01% to 0.04% and duration of exposure from several seconds to 4 or 5 minutes in eyes with exceptionally high risk of failure.<sup>[2]</sup> It appears that the rate of MMC related complications is associated with both the concentration and duration of exposure to this agent.<sup>[4]</sup>

Decreased corneal endothelial cell density (CECD) is another recognized adverse effect associated with MMC use and has been evaluated in a number of ocular surface procedures including excimer laser refractive surgery, treatment of ocular surface neoplasia, and pterygium surgery. CECD is known to stabilize by 3–6 months following exposure to MMC or mechanical stress to the endothelium; corneal endothelial repopulation and migration is believed to compensate for CECD loss in affected areas.

CECD loss associated with glaucoma surgery has been largely unexplored. MMC can be applied before or after scleral flap dissection; the latter sequence has been associated with increased surgical success rates.<sup>[5-8]</sup> Theoretically, intraocular penetration of MMC and hence possible endothelial cell damage, may be facilitated if the agent is applied after scleral flap dissection. The current study was designed to evaluate changes in corneal endothelial cell density following conventional trabeculectomy comparing two different methods of MMC application, that is, before versus after scleral flap dissection. We also compared success rates for these two surgical sequences.

## METHODS

### Setting

This double-masked, randomized clinical trial included patients undergoing primary trabeculectomy for poorly controlled glaucoma at Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran and was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT01297803.

### Inclusion and Exclusion Criteria

Subjects 16–80 years of age with juvenile open angle glaucoma (JOAG), primary open angle glaucoma (POAG), primary angle closure

glaucoma (PACG) and pseudoexfoliation glaucoma (PXG) who were scheduled for primary trabeculectomy were evaluated for enrollment. Eyes with any preexisting corneal endothelial abnormality, history of trauma or intraocular surgery were excluded. Eyes with congenital glaucoma, secondary glaucomas such as active uveitis, neovascular glaucoma and those associated with conditions such as aniridia, axenfeld-rieger, iridocorneal endothelial and Peters syndromes were also excluded.

### Informed Consent and Ethical Issues

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee at the Ophthalmic Research Center; written informed consent was obtained from all subjects or their legal guardians prior to enrollment.

### Preoperative Evaluations

All eligible subjects were interviewed and underwent a comprehensive ophthalmological examination including slit lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy and fundus examination. Specular microscopy was performed using the Tomey EM-3000 (Tomey corp, Nagoya, Japan) at the center of the cornea by a masked technician. Corneal endothelial cell density, polymegathism and pleomorphism were measured before surgery and 1, 3 and 6 months after trabeculectomy.

### Randomization and Masking

Eyes were randomly assigned to MMC application before (Group A) or after (Group B) scleral flap dissection according to the duration of MMC application (1, 2, or 3 min). Randomization was stratified according to MMC application time to avoid the confounding effect of this parameter. The same concentration of MMC (0.02%) was used in all eyes.<sup>[9,10]</sup> All patients, the ophthalmologist who examined the patients and recorded postoperative data, the technician in charge of specular microscopy and the statistician involved in data analysis were unaware of patient identity and study group.

### Intervention

Trabeculectomy was performed in the same manner by two experienced glaucoma specialists (SY and MP) in all cases. A superonasal or superotemporal fornix based conjunctival peritomy was performed followed by dissection of subconjunctival tissues and gentle hemostasis using wet-field bipolar cautery. At this stage the surgeon would choose the duration of MMC application based on factors such as patient age, type of glaucoma, level of glaucomatous damage,

and conjunctival vascularity and texture. MMC was applied at a constant concentration of 0.02% in all eyes using 4–5 pieces of soaked cellulose sponges under the whole area of conjunctival dissection; depending on randomization the surgeon would apply MMC under the conjunctiva flap at this stage or leave it until scleral flap dissection was completed. The scleral flap was trapezoidal, 3.5 mm at its base, approximately half scleral thickness and dissected into clear cornea in all cases. Placement of MMC-soaked sponges was identical in both groups meaning that in Group B, MMC was simply allowed to seep directly under the scleral flap without actually placing a piece of sponge directly under the flap. Next preplaced 10/0 nylon releasable sutures were placed at the corners of the scleral flap; this stage was followed by a paracentesis, internal block excision and a peripheral iridectomy. The scleral flap sutures were tied using 4 throws without locking the knot and filtration was titrated to reach a desirable endpoint of balanced filtration. Finally the conjunctiva was repaired using 10/0 nylon sutures.

### Outcome Measures

The main outcome measure was the percentage of change in endothelial cell density after the procedure; secondary outcome measures included IOP levels, the number of medications, success rates and complications. Complete success was defined as  $6 < \text{IOP} \leq 21$  mmHg (criterion 1) and  $6 < \text{IOP} \leq 18$  mmHg (criterion 2) without using glaucoma medications, partial success was defined as the same on a maximum of 2 medications, and total success was considered as the sum of complete and partial success rates. Failure was defined as  $\text{IOP} > 21$  or 18, and  $\text{IOP} \leq 21$  or 18 mmHg using more than 2 topical medications, complete loss of vision, and reoperation for further IOP control. IOP levels, medications, success rates and complications were recorded at each follow-up visit; the minimum early postoperative follow-up schedule included days 1, 3, 7, 14, and 28 but outcomes of the study were assessed 1, 3 and 6 months after surgery. Additional examinations were scheduled depending on the clinical condition and if complications were encountered. Study outcomes were evaluated and recorded by a masked ophthalmologist.

### Sample Size and Statistical Methods

Sample size was calculated based on changes in endothelial cell density 6 months after the procedure. Based on a pilot study, the standard deviation (SD) of the percentage of change in endothelial cell density was 8 in both groups. We therefore required 54 eyes in each study group to be able to detect a difference of 5% in CECD loss between the groups with power of 90%.

Mean, SDs, and percentages were used to describe the study parameters. T-test, and Mann–Whitney,

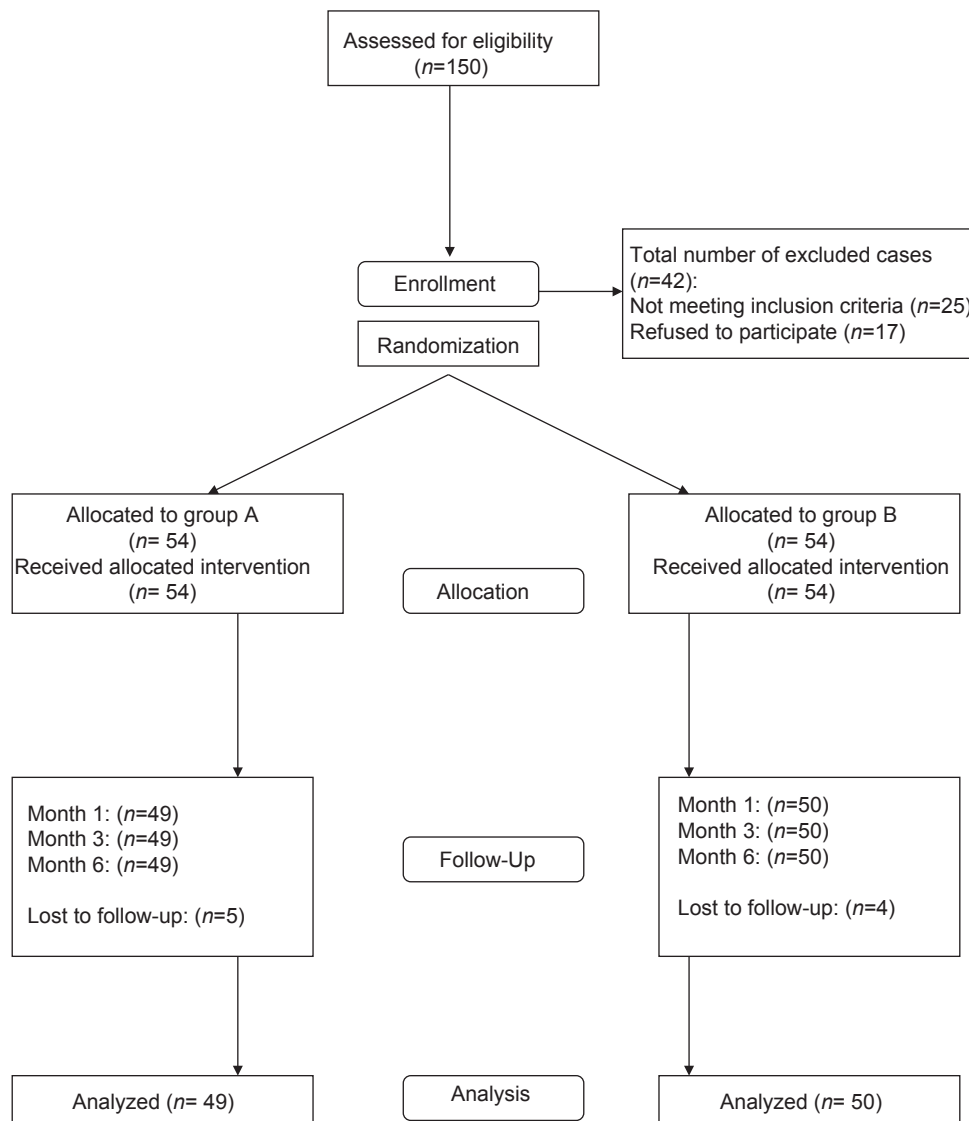
Fisher's exact, and Chi-Square tests were used to compare parameters between the study groups. Analysis of Covariance was used to adjust for baseline effect. Repeated measure analysis with a Bonferroni correction for multiple comparisons was applied to evaluate changes within the groups. All statistical analyses were performed by a masked biostatistician using SPSS software version 17.0 (SPSS co, Chicago, IL, USA); the group label was uncovered after the analysis was completed.

## RESULTS

Initially 150 patients were assessed for eligibility; after applying the study criteria and obtaining informed consent, 108 eyes of 108 patients were enrolled. All eligible subjects received the allocated intervention. Nine cases consisting of 6 female and 3 male subjects, including 5 patients in Group A and 4 others in Group B, were lost to follow-up and thus eliminated from the analysis. Loss to follow-up rate was 10% and less than anticipated. Eventually 99 eyes of 99 subjects who completed the 6-month follow-up period were analyzed; these included 49 and 50 eyes in groups A and B respectively [Figure 1]. The study groups were comparable in terms of age, sex, type of glaucoma, number of glaucoma medications, mean IOP and duration of MMC application [Table 1]. Baseline corneal endothelial cell parameters were comparable between the study group in terms of cell density, pleomorphism and polymegathism [Table 1].

Corneal endothelial cell reduction was comparable between Groups A and B at all postoperative visits [Table 2]. The lowest corneal endothelial cell density (CECD) was observed one month after surgery in both groups; thereafter CECD was slightly increased at 3 and 6 months but did not reach preoperative values. CECD was significantly reduced from baseline values in Group A only 1 month after surgery ( $P < 0.01$ ) but not at 3 ( $P = 0.06$ ) and 6 months ( $P = 0.1$ ). In Group B, CECD was also significantly reduced only 1 month ( $P = 0.02$ ) after surgery but not at 3 ( $P = 0.44$ ) and 6 ( $P = 0.95$ ) months.

The study groups were comparable in terms of corneal endothelial cell polymegathism at months one ( $P = 0.98$ ), 3 ( $P = 0.77$ ), and 6 ( $P = 0.54$ ). We observed a trend of increasing polymegathism in Group A at month 1 ( $P = 0.07$ ) which became significant at month 3 ( $P = 0.02$ ); although this value decreased, it failed to return to preoperative values but the difference from baseline was not significant at 6 months ( $P = 0.27$ ). In Group B, increased polymegathism was statistically significant 1 month after surgery ( $P = 0.03$ ) and although still higher than preoperative values, the index was not significantly higher at months 3 ( $P = 0.29$ ) and 6 ( $P = 0.79$ ).



**Figure 1.** Flow of the study participants at different stages of the study.

Corneal endothelial cell pleomorphism was significantly reduced in Group A 1 month after surgery ( $P = 0.04$ ) and although still lower than preoperative values, differences were not significant at 3 ( $P = 0.61$ ) and 6 ( $P = 0.74$ ) months. In Group B, no significant change was observed in pleomorphism at months 1 ( $P = 0.49$ ), 3 ( $P > 0.99$ ), and 6 ( $P > 0.99$ ). In terms of changes in pleomorphism, Groups A and B were comparable at months 1 ( $P = 0.08$ ) and 3 ( $P = 0.27$ ), however at 6 months the percentage of hexagonal cells was significantly lower in Group A as compared to Group B ( $P = 0.05$ ) in which hexagonality had apparently exceeded preoperative values.

IOP was significantly reduced after surgery within both groups at all postoperative time points ( $P < 0.01$  for all comparisons) and no significant difference was observed in absolute IOP levels or percentage of IOP reduction between Groups A and B at any time interval [Table 3].

The mean number of glaucoma medications was  $2.8 \pm 1$  and  $2.7 \pm 0.9$  ( $P = 0.47$ ) before surgery in Groups A and B respectively. The majority of eyes were off glaucoma medications at all postoperative visits and the study groups were comparable in this regard ( $0.1 \pm 0.3$  vs. nil [ $P = 0.15$ ],  $0.2 \pm 0.5$  vs.  $0.1 \pm 0.3$  [ $P = 0.68$ ] and  $0.2 \pm 0.6$  vs.  $0.1 \pm 0.4$  [ $P = 0.45$ ] at months 1, 3 and 6, respectively).

No significant difference was observed between Groups A and B regarding complete and partial success rates at any time interval. Overall success rates were comparable between the study groups at 1 and 3 months [Figures 2 and 3], but significantly higher in Group B at 6 months using both IOP success criteria (63.3% vs. 82.0%,  $P = 0.036$  for IOP  $\leq 21$  mmHg and 59.2% vs. 78.0% for IOP  $\leq 18$  mmHg,  $P = 0.044$  in Groups A and B respectively, Tables 4 and 5).

The study groups were comparable in terms of complications; 14.2% of eyes in Group A and 16% of

**Table 1. Baseline clinical data**

	Total	Group A	Group B	P
Age	60.54±13.19	60.41±12.66	60.66±13.71	0.925 <sup>†</sup>
Medications	2.77±0.93	2.84±0.96	2.7±0.9	0.475 <sup>‡</sup>
IOP	19.4±7.11	18.43±6.27	20.36±7.74	0.179 <sup>†</sup>
Sex (%)				
Male	72 (72.7)	33 (67.3)	39 (78.0)	0.234 <sup>*</sup>
Female	27 (27.3)	16 (32.7)	11 (22.0)	
Glaucoma type (%)				
POAG	32 (32.3)	18 (36.7)	14 (28.0)	0.455 <sup>**</sup>
PXG	5 (5.1)	1 (2.0)	4 (8.0)	
PACG	52 (52.5)	26 (53.1)	26 (52.0)	
JOAG	10 (10.1)	4 (8.2)	6 (12.0)	
Endothelial cell density	2290.69±367.54	2292.73±320.83	2288.68±408.99	0.957 <sup>†</sup>
Polymegathism	38.15±7.11	37.94±6.46	38.36±7.7	0.771 <sup>†</sup>
Polymorphism	48.54±9.19	49.73±6.9	47.36±10.88	0.202 <sup>†</sup>
MMC time (min) (%)				
1	14 (14.1)	9 (18.4)	5 (10.0)	0.160 <sup>‡</sup>
2	74 (74.7)	36 (73.5)	38 (76.0)	
3	11 (11.1)	4 (8.2)	7 (14.0)	

<sup>†</sup>Based on *t*-test. <sup>\*</sup>Based on Mann-Whitney test. <sup>\*</sup>Based on Chi-square test, <sup>\*\*</sup>Based on Fisher exact test. POAG, primary open angle glaucoma; PXG, pseudoexfoliative glaucoma; PACG, primary angle closure glaucoma; JOAG, juvenile open angle glaucoma; MMC, mitomycin-C; IOP, intraocular pressure

**Table 2. Corneal endothelial cell density before, one, 3 and 6 months after surgery**

	A	B	Mean difference (95% CI)	P
Preoperative				
Value (mean±SD)	2292.73±323.33	2288.68±412.11	4.05 (-143.9-152.01)	0.957 <sup>†</sup>
Month 1				
Value (mean±SD)	2202.94±322.26	2210.94±392.22	-8 (-151.35-135.35)	0.708 <sup>‡</sup>
Change	89.8±153.65	77.74±163.74	12.06 (-51.31-75.42)	
Change %	3.78±7.01	3.09±7.55	0.69 (-2.22-3.6)	0.497 <sup>‡</sup>
<i>P</i> <sup>‡</sup>	0.001	0.017		
Month 3				
Value (mean±SD)	2218.35±303.19	2245.24±407.08	-26.89 (-170.3-116.51)	0.370 <sup>‡</sup>
Change	74.39±193.37	43.44±154.61	30.95 (-38.82-100.71)	
Change %	2.81±8.36	1.71±6.99	1.1 (-1.97-4.17)	0.823 <sup>‡</sup>
<i>P</i> <sup>‡</sup>	0.058	0.438		
Month 6				
Value (mean±SD)	2233.22±305.19	2255.43±405.66	-22.2 (-166.15-121.75)	0.317 <sup>‡</sup>
Change	59.51±169.82	28.18±137.45	31.33 (-30.63-93.28)	
Change %	2.25±7.32	0.99±6.34	1.26 (-1.48-4.01)	0.567 <sup>‡</sup>
<i>P</i> <sup>‡</sup>	0.107	0.946		

<sup>†</sup>Based on repeated measure analysis, adjusted for multiple comparison by Bonferroni correction. <sup>‡</sup>Based on *t*-test. <sup>‡</sup>Based on Mann-Whitney test. <sup>‡</sup>Based on ANCOVA. ANCOVA, analysis of covariance; SD, standard deviation; CI, confidence interval

those in Group B ( $P = 0.78$ ) developed a significant complication [Table 6]. Insignificant complications such as transient leaks and spontaneously improving shallow anterior chambers are not reported. Although hypotony was more prevalent with after-the-flap MMC application (2% vs. 8% in Groups A and B), this difference failed to reach statistical significance ( $P = 0.38$ ).

## DISCUSSION

The rationale behind MMC application after dissection of the scleral flap during trabeculectomy is to increase success by augmenting the antifibrotic effect of the agent.<sup>18</sup> Drawbacks to this practice, at least theoretically, include facilitated penetration of MMC into the anterior chamber across the partial-thickness corneoscleral bed



predisposing to greater corneal endothelial cell damage and a higher risk of ciliary body toxicity and hypotony; these concerns formed the basis of the current research.

To our knowledge this is the first study designed to compare corneal endothelial cell density, pleomorphism, and polymegathism following trabeculectomy using 2 methods of MMC application. Our RCT demonstrated comparable safety in terms of endothelial damage with these 2 different methods of MMC application, that is, before and after dissection of the scleral flap. We avoided direct application of MMC soaked sponges under the scleral flap and performed our routine surgical practice by applying MMC in the same manner in both groups allowing seepage of the antifibrotic agent underneath the scleral flap in Group B. Adverse effects of MMC could be dose dependent; we therefore used the same concentration (0.02%) of MMC in all eyes and also

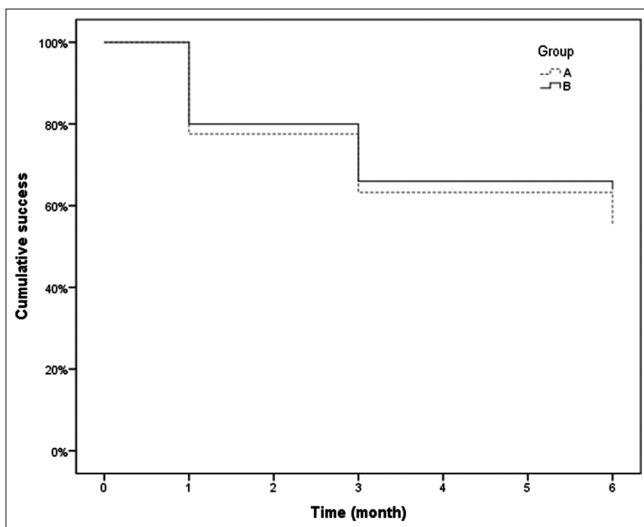
stratified eligible eyes based on the duration of MMC application prior to randomization to equally distribute eyes with different durations of use (i.e., 1–3 min) into the study subgroups.

We observed no significant difference between our study groups in terms of CECD and polymegathism at any time interval, although pleomorphism was slightly better in Group B (MMC application after scleral flap dissection) at 6 months. Decreased CECD and increased polymegathism which were observed at month 1 in both groups is compatible with initial stress and damage to the endothelium followed by compensatory enlargement and migration of residual cells in order to repopulate the damaged areas; this notion was reflected by improved cell density and reduced polymegathism at months 3 or 6, although these parameters failed to reach baseline values. Hexagonality was comparable between the 2 groups and reduced from baseline at month 1, a finding in line with the pattern of damage mentioned above. However the intergroup difference in hexagonality in favor of Group B at months 6, which was even higher than baseline values, is difficult to account for; causes for this observation may include instrument or measurement variability because one cannot expect an improvement in the percentage of hexagonal cells following intraocular surgery. A single study on 14 cases by Storr-Paulsen et al. reported significantly decreased CECD 3 months after trabeculectomy augmented with MMC but the change was not significant from month 3 to 12.<sup>[11]</sup> Similar to our study, these authors measured CECD changes in the center of the cornea and our findings are in line with the pattern of change they observed. Other studies on CECD changes after intraocular surgery have demonstrated that surgically induced changes, usually stabilize 6 months after intervention.<sup>[12]</sup>

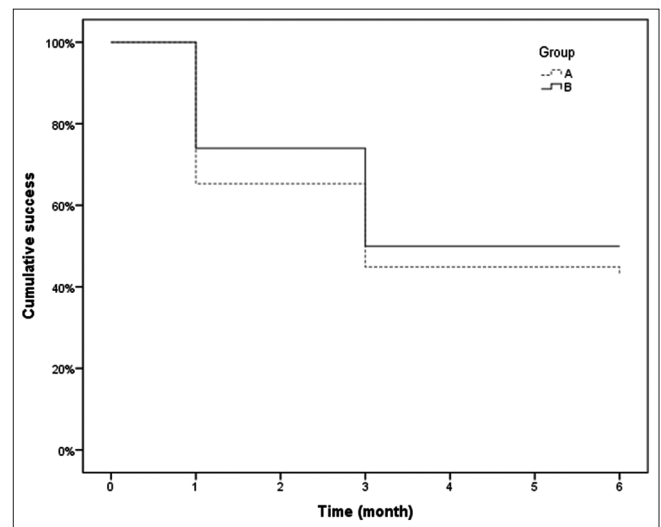
**Table 3. IOP before and at months 1, 3, and 6 after surgery**

Time	Group A	Group B	P
Before surgery	18.4±6.3	20.4±7.8	0.179 <sup>†</sup>
Month 1	11.9±5.5	12.1±5.1	0.903 <sup>‡</sup>
Reduction %	30.2±33.3	34.7±30.3	0.484 <sup>‡</sup>
P <sup>€</sup>	<0.001	<0.001	
Month 3	11.1±5.5	11.7±5.0	0.614 <sup>‡</sup>
Reduction %	34.4±33.2	35.1±36.5	0.922 <sup>‡</sup>
P <sup>€</sup>	<0.001	<0.001	
Month 6	11.8±5.8	11.7±5.5	0.878 <sup>‡</sup>
Reduction %	30.0±37.0	36.0±37.4	0.425 <sup>‡</sup>
P <sup>€</sup>	<0.001	<0.001	

<sup>€</sup>Based on repeated measure analysis, adjusted for multiple comparison by Bonferroni correction. <sup>†</sup>Based on *t*-test. <sup>‡</sup>Based on Mann-Whitney test. <sup>‡</sup>Based on ANCOVA. ANCOVA, analysis of covariance; IOP, intraocular pressure



**Figure 2.** Kaplan–Meier survival graph for cumulative success during the study period according to criterion 1 (intraocular pressure [IOP] <21 and >8 mmHg) in the study groups (log-rank test, *P* = 0.411).



**Figure 3.** Kaplan–Meier survival graph for cumulative success during the study period according to criterion 2 (IOP <18 and >8 mmHg) in the study groups (log-rank test, *P* = 0.416).

**Table 4. Complete, partial, and total success rates (IOP ≤ 21 mmHg)**

Time	Status	Group A	Group B	P**
Month 1 (%)	Complete	37 (75.5)	39 (78.0)	0.594*
	Partial	1 (2.0)	0 (0.0)	
	Overall	38 (77.6)	39 (78.0)	
Month 3 (%)	Complete	31 (63.3)	37 (74)	0.452**
	Partial	3 (6.1)	3 (6.0)	
	Overall	34 (69.4)	40 (80.0)	
Month 6 (%)	Complete	29 (59.2)	38 (76.0)	0.124**
	Partial	2 (4.1)	3 (6.0)	
	Overall	31 (63.3)	41 (82.0)	

\*Based on Chi-square test. \*\*Based on Fisher exact test. IOP, intraocular pressure

**Table 5. Complete, partial, and total success rates (IOP ≤ 18 mmHg)**

Time	Status	Group A	Group B	P*
Month 1 (%)	Complete	32 (65.3)	37 (74.0)	0.347
	Partial	0 (0.0)	0 (0.0)	
	Overall	32 (65.3)	37 (74.0)	
Month 3 (%)	Complete	31 (63.3)	36 (72.0)	0.353
	Partial	2 (4.1)	2 (4.0)	
	Overall	33 (67.3)	38 (76.0)	
Month 6 (%)	Complete	28 (57.1)	37 (74.0)	0.077
	Partial	1 (2.0)	2 (4.0)	
	Overall	29 (59.2)	39 (78.0)	

\*Based on Chi-square test. IOP, intraocular pressure

**Table 6. Postoperative complications in Groups A and B**

Complication	Group A (%)	Group B (%)	Total (%)
None	42 (85.8)	42 (84)	84 (84.8)
Persistent hypotony	1 (2.0)	2 (4.0)	3 (3.0)
Hypotony maculopathy	0 (0)	2 (4.0)	2 (2.0)
Choroidal effusion leading to drainage	2 (4.1)	2 (4.0)	4 (4.0)
Bleb-associated endophthalmitis	1 (2.0)	0 (0)	1 (1.0)
Bleb leakage requiring resuturing	3 (6.1)	2 (4.0)	5 (5.1)

Net IOP change and the percentage of IOP reduction was similar in both groups at all-time points. The number of anti-glaucoma medications was significantly reduced 1, 3, and 6 months after surgery in both groups and there was no significant difference between them in this regard. No significant difference was observed between the study groups in terms of complete and partial success rates at any follow-up interval. But overall success rate was higher in Group B at 6 months. This suggests a benefit from applying MMC after scleral flap dissection in terms of success rates. Other studies addressing success rates following trabeculectomy have had conflicting results; 3

groups of investigators have reported no difference with these 2 methods of MMC application, while 2 studies have demonstrated better success rates when MMC is applied after scleral flap dissection [Table 7].

Tressler et al.,<sup>[13]</sup> Vass et al.,<sup>[14]</sup> and Agarwal et al.<sup>[15]</sup> reported no superiority with intrascleral MMC application while the studies by El Sayyad et al.<sup>[7]</sup> and Prata et al.<sup>[16]</sup> have revealed improved outcomes with subscleral MMC application. The cause of greater IOP reduction with MMC application under both flaps may be simultaneous inhibition of Tenon’s fibroblasts, and those causing scleral flap fibrosis. Furthermore MMC application under the scleral flap can be associated with greater intraocular penetration of the agent thereby reducing aqueous production by the ciliary body; this may also be the cause of the higher rate of hypotony we observed.

Our study groups were comparable in terms of the overall rate of significant complications or those requiring intervention; some conditions were observed with almost the same frequency with both methods of MMC application including bleb leaks leading to resuturing, and choroidal effusions requiring drainage. El Sayyad et al.<sup>[7]</sup> and Agarwal et al.<sup>[15]</sup> also found no significant difference in complication rates with subconjunctival and subscleral/intrascleral MMC application. In contrast Tressler et al.<sup>[13]</sup> reported a greater need for posttrabeculectomy procedures with intrascleral MMC application. The latter study was performed applying MMC for 5 min, while in our study the maximum duration of application was 3 min. The higher duration of MMC application may be the cause of more posttrabeculectomy procedures required with the intrascleral method in Tressler’s study.

Although in our study the rate of persistent hypotony and hypotony maculopathy with after-the flap MMC application was 4 times higher, this difference failed to reach statistical significance which is probably due to the small number of eyes with this condition. Facilitated MMC penetration after scleral flap dissection may be the cause of the higher rate of hypotony and hypotony maculopathy in Group B; a condition similar to that observed in myopic eyes, in which a thin sclera may predispose to hypotony maculopathy.<sup>[15]</sup> We observed one case (2%) of bleb associated endophthalmitis in Group A which fortunately responded to intravitreal and intravenous antibiotics. This eye with POAG developed a thin cystic bleb following only 1 minute of MMC application before scleral flap dissection. The incidence of bleb related infections and bleb-associated endophthalmitis in large collaborative studies or those enrolling more than 1000 cases have revealed infections rates ranging from 0.45% to 5%.<sup>[17-20]</sup>

In summary, this study demonstrated comparable corneal endothelial cell loss following trabeculectomy

**Table 7. Comparison of similar studies on MMC application in trabeculectomy**

	Year of study	Study design	Number of cases	Follow-up	Remarks	Complications
Vass et al <sup>[14]</sup>	1998	Retrospective	38 eyes 29 patients	19 months 24 months sandwich	No significant difference	No difference
Tressler et al <sup>[13]</sup>	1996	Prospective randomized	24 eyes 23 patients		More IOP lowering procedures required with MMC application after scleral flap	More in the intrascleral group
Agarwal et al <sup>[15]</sup>	2001	Randomized prospective clinical study	41 eyes	1-year	Lower IOPs in the early postoperative period but no significant difference in IOP or success rates 90.5% subconjunctival 75% intrascleral	More hypotony with intrascleral application but not clinically significant
El Sayyad et al <sup>[7]</sup>	2000	Prospective randomized study	68 eyes	12 months	Lower IOP with MMC under both flaps but no significant difference in success rates 77% Subconjunctival 71% subscleral	No difference
Prata et al <sup>[16]</sup>	1994	Retrospective	82 eyes 82 patients	10 months	success rates 50% episcleral 93.5% scleral	No difference
Yazdani et al	2013	Randomized clinical trial	99 eyes 99 patients	6 months	success rates 63.3% before flap 82% after flap	More hypotony with intrascleral application but not clinically significant

MMC, mitomycin-C; IOPs, intraocular pressures

using 2 methods of MMC application, that is, before or after dissection of the scleral flap. Overall success rate was higher with after-the-flap MMC usage at 6 months but both sequences were associated with comparable levels of IOP reduction and similar rates of complications. Although hypotony was more common with MMC application after scleral flap dissection, the observed difference was not statistically significant.

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