Original Article

Evaluation of Corneal Endothelial Cells and Morphology in Mitomycin-C Augmented Trabeculectomy

Shalini Dhiman, Ankur Kumar, Kishor Kumar
SMS Medical College, Jaipur, Rajasthan, India

Abstract

Purpose: *To evaluate the effect of Mitomycin-C (MMC) on corneal endothelial cells and morphology in Mitomycin-C augmented trabeculectomy*

Methods: *In this study, 40 eyes of patients underwent trabeculectomy with Mitomycin-C (group 1) and 40 eyes of patients underwent trabeculectomy without Mitomycin-C (group 2). Specular microscopy was performed at 1 month and 3 month postoperatively. Outcome variables included corneal endothelial cell density (CECD), coefficient of variation of cell size (CV), central corneal thickness (CCT), hexagonality.*

Results: *Overall, mean preoperative CECD was 2538.21+/-259.98 mm^2, postoperatively at 1 month and 3 month it was significantly reduced to 2377.11+/-270.36 mm^2 and 2340.56+/-272.39 mm^2 respectively. (p <0.05). Overall, mean preoperative CV was 31+/-5.93%, postoperatively at 1 month and 3 month it was non-significantly increased to 33.19+/-6.02% and 34.30+/-5.97% respectively. (p>0.05). Overall, mean preoperative hexagonality was 53.63+/-4.47%, postoperatively at 1 month and 3 month it was non significantly reduced to 51.54+/-4.50% and 50.41+/-4.49% respectively (p>0.05). Overall, mean preoperative CCT was 514.41+/-13.786um, postoperatively at 1 month and 3 month it was significantly increased to 523.58+/-14.99um and 520.69+/-14.80 um respectively. (p<0.05).*

Conclusion: *Mitomycin-C application in trabeculectomy cause significant corneal endothelial loss. Most of the damage occurs intraoperatively, or in the early postoperative period.*

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Keywords: *MMC, Trabeculectomy,CECD.*

Introduction

Glaucoma is a serious sight-threatening disorder aptly named the Silent thief of Sight. It is the second leading cause of blindness in India and the country has been predicted to host nearly 20% of the world glaucoma population by 2020.[1,2] Trabeculectomy is the most commonly performed surgical treatment in dealing with Primary open angle glaucoma worldwide. The most common cause of failure of glaucoma filtering procedure is wound healing with scarring of the outflow area. Adjunctive antifibrotic agents, such as 5-fluorouracil (5-FU) or Mitomycin-C (MMC), are commonly used to increase the success rate of glaucoma filtering surgery.[3,4]

MMC is an antibiotic derived from Streptomyces caespitosus with alkylating properties, has an inhibitory effect on fibrosis and vascular growth both of which play important roles in tissue healing and scar formation. In trabeculectomy, MMC may penetrate into adjacent ocular tissues, beyond its application site.[5] Since corneal endothelial cells lack division capacity possible damage are irreparable and cell density diminishes gradually.[6,7]

Specular microscopy is used to evaluate the health of the corneal endothelium or inspect any damage that may have been caused to it by disease, surgery or injury.

The present study was undertaken to assess and compare the effect of Mitomycin-C on corneal endothelial cells in Mitomycin-C augmented trabeculectomy and standard trabeculectomy.

Methods

This prospective randomized comparative case study included patients scheduled for trabeculectomy at the upgraded department of ophthalmology, SMS medical college & hospitals, Jaipur. 80 adult patients were enrolled in the study. After explaining the study, surgical procedures and possible complications, an informed consent was obtained and patients were assigned to two groups;

Group 1 (n = 40) who underwent trabeculectomy with intraoperative application of 0.2 mg/ml Mitomycin-C for 2 minutes

Group 2 (n = 40) who underwent standard trabeculectomy.

Eligibility Criteria

Inclusion criteria:

Patients with primary open angle glaucoma (POAG), who either, despite receiving maximal tolerable medical treatment, had higher than target IOP, who were intolerant to medications, Patient who will give written informed consent were included in study.

Exclusion criteria

Angle closure glaucoma, Secondary glaucomas other than PXFG, Previous intraocular surgery or laser procedures, Performing cataract surgery simultaneously or during the follow-up period, Intraocular disorders other than mild cataracts,Postoperative flat anterior chamber,

Endophthalmitis, Marked postoperative inflammation, Intraoperative choroidal hemorrhage, any corneal endothelial pathology were excluded from study.

Pre-operative evaluation:
Baseline information, such as, age, gender, number of anti-glaucoma medications and medical history were recorded. All patients received a complete preoperative examination, including best corrected visual acuity measurement (Snellen chart), slit lamp examination, tonometry (Goldmann applanation tonometry), gonioscopy, dilated fundus examination, a Humphrey visual field (24-2, or 30-2) examination, and specular microscopy

Surgical technique:
All surgeries were performed by the same surgeon under peribulbar anesthesia.
In group 1, Superior rectus bridle suture was placed, a fornix-based conjunctival flap prepared (Figure 1), hemostasis was achieved by adequate wet-field cautery, and; 4 mm × 4 mm rectangular scleral flap one-third of the thickness dissected to within 1 mm of clear cornea with a bard-parker knife (Figure 2a & 2b). MMC 0.2 mg/ml was applied for 2 min beneath conjunctival flap and sclera flap (Figure 3). Surgical area was irrigated with BSS. After creating a paracentesis opening, inner sclerostomy block was dissected out with the blade in the dimensions 2mm × 3mm, at the base of the hinge of the superficial scleral flap (Figure 4). Peripheral iridectomy performed through the inner sclerostomy with a Vannas scissor and a single-toothed fine forceps (Figure 5). Scleral flap reapproximated with two 10-0 nylon suture, conjunctival flap closed watertight by running 10-0 nylon suture (Figure 6 & 7). Subconjunctival injection of 0.3 ml gentamycin and 0.3 ml dexamethasone was given, completing the procedure.
Group 2 patients underwent the same procedure without intraoperative application of MMC.
Postoperatively, patients were prescribed a combination of antibiotic-steroid (tobramycin 0.3% + dexamethasone 0.1%) eye drops every 2 h for 1 week which tapered over the following 5 weeks. Cycloplegic-mydriatic (homatropine 2%) eye drops were used when signs of early inflammation appeared and shallow A/C or hypotony was present.
Follow up examination was conducted 1st and 3rd day, 1 week and 2 week, 1 month and 3 month postoperatively with specular microscopy done at 1st and 3rd month postoperatively. Three specular photographs were taken at every examination, and the mean data were considered for statistical analysis.

Statistical analysis:
Statistical analysis was performed using Statistical Package for Social Sciences software version 23 (SPSSInc., Chicago, Illinois, USA). Analysis of variance (ANOVA) was used to analyze intragroup changes in continuous variables pre and postoperatively. In cases of Normal distribution of data, mean and SD were used while in cases variable are not normally distributed then median were used. The Mann Whitney U test was used to compare mean values of intergroup

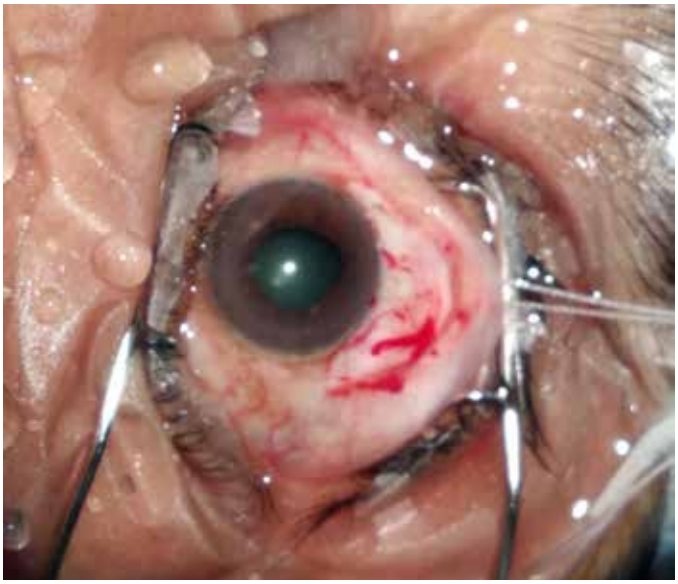

Figure 1: conjunctival peritomy done fornix-based conjunctival flap made

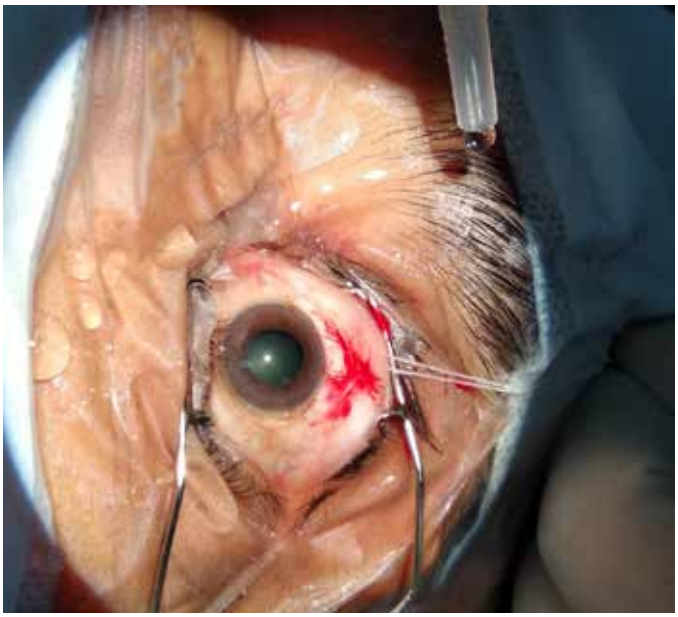

Figure 2a: partial thickness scleral flap made

Figure 2b: partial thickness scleral flap

Figure 3: Mitomycin-c applied under conjunctival and scleral flap for 2 minutes

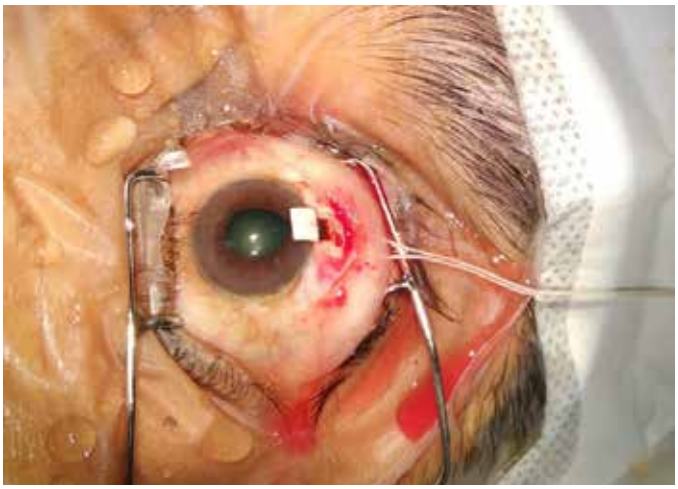

Figure 4: sclerostomy made

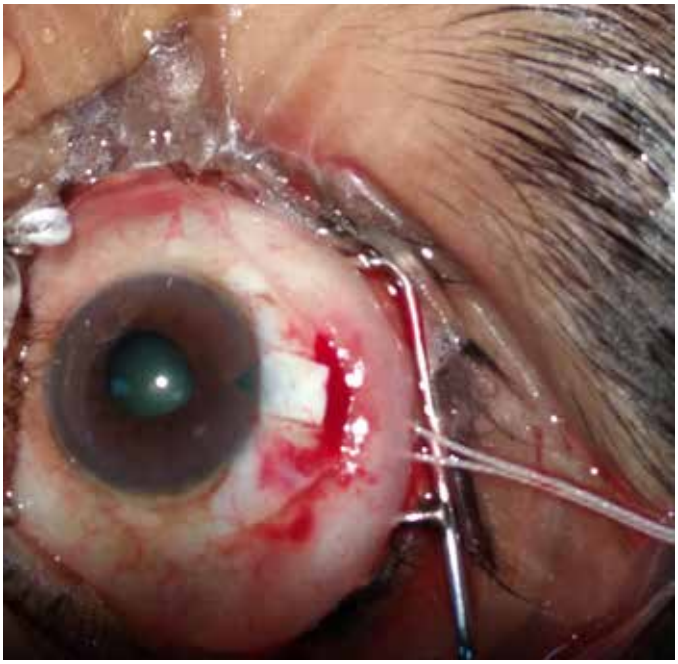

Figure 5: peripheral iridectomy done

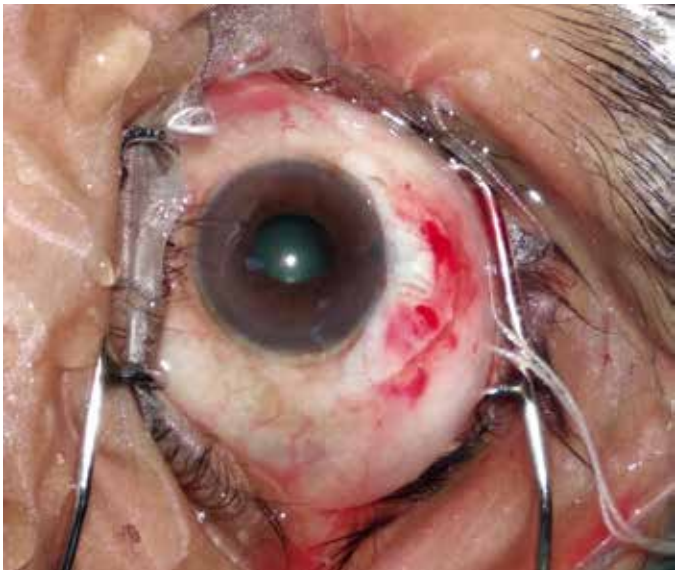

Figure 6: scleral flap sutured with 10-0 nylon suture

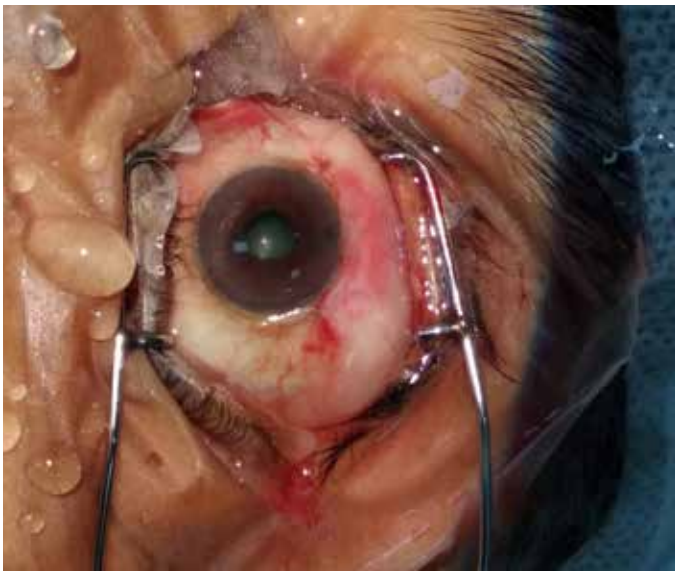

Figure 7: conjunctival flap sutured with 10-0 nylon suture

continuous variables. Categorical data was evaluated using the Chi square test. Wilcoxon one sample nonparametric test was used to compare the means of intragroup continuous variables. For all measurements, a two tailed test was used, and $P < 0.05$ was considered as significant for measured variables.

Results

80 eyes of 80 patients were evaluated in our study with the aim to study and assess the corneal endothelial cell density, coefficient of variation of cell area, hexagonality, central corneal thickness in patients undergoing trabeculectomy with Mitomycin-C (GROUP 1) and trabeculectomy without Mitomycin-C (GROUP 2).

Our study reports postoperative status of the corneal endothelium after trabeculectomy and compares the status of the 2 groups. In our study, the mean age was

55.20±9.819 years in Group 1 and 55.43±11.001 years in Group 2. There were no significant differences in terms of mean age (P=0.92NS). No significant difference was observed according to gender i.e groups were comparable according to gender. (P=0.482). No significant difference were observed in CECD, CV, Hexagonality and CCT preoperatively. i.e groups were comparable.

Patients' demographic and baseline characteristics are detailed in Table 1.

Table 1: Demographic and baseline characteristics

	Group 1 (n=40)	Group 2 (n=40)	P value	Overall (n =80)
Mean Age (years)	55.20 +/- 9.82	55.43+/-11.0	0.92	55.31+/-10.36
Female (%)	16:40 (40%)	12:40 (30%)	0.482	28
Preoperative CECD (mm^2)	2489.48+/- 244.639	2586.95+/- 268.680	0.094	2538.21+/- 259.77
Preoperative Hexagonality (%)	54.23 +/- 4.588	53.03 +/- 4.329	0.233	53.63+/-4.473
Preoperative CV (%)	30.20 +/- 6.362	31.80 +/- 5.426	0.230	31.00+/-5.930
Preoperative CCT (um)	515.75 +/- 13.44	513.08+/- 14.161	0.38	514.41+/- 1.00

This study assessed the endothelial cell count, post-operatively at intervals of 1 month and 3 months and noted that, In Group 1, the mean endothelial cell count dropped to 2290.65±245.08/mm^2 at 1 month (P=0.004) and 2248.78±243.06/mm^2 at 3 months. (P=0.002). In Group 2, the mean corneal endothelial cell count dropped to 2463.58 ± 269.55/mm^2 at 1 month and 2432.35±271.90/mm^2 at 3 months. It was observed that pre-operatively there was no significant difference in the endothelial cell counts between the two groups, still the mean endothelial cell loss was significantly higher in group 1 as compared to group 2 at each follow up. (P=0.004).

The percentage of cell loss from baseline to postoperative month 1 in group 1 versus 2 was 7.98% and 4.76%, respectively (P=0.004). CECD loss from baseline to postoperative month 3 in group 1 versus 2 was 9.66% and 5.97%, respectively (P=0.002). CECD loss from months 1 to 3, in group 1 versus 2 was 1.68% and 1.21%, respectively. (Table 2) (Figure 8)

After the surgery the mean percentage of CV increased in both the groups. In group 1 it was increased to 32.33 ± 6.39% at 1 month and 33.55 ± 6.25% at 3 months. In group 2 it was Increased to 34.05 ± 5.57% at 1 month and 35.05 ± 5.67% at 3 months. Though the mean percentage of CV gradually increased in both the groups, but it was not statistically significant. (P=0.264). (Table 3) (Figure 9).

After the surgery the mean percentage of hexagonality dropped in both the groups. In group 1 it was reduced to 51.70 ± 4.65% at 1month and 50.40 ± 4.71%at 3 months. In group 2 it was reduced to 51.38 ± 4.40% at 1 month and 50.43 ± 4.33% at 3 months. Though the mean percentage of hexagonality gradually decreased in both the groups, but it was not statistically significant (P=0.98) (Table 4) (Figure 10).

Table 2: CECD (per sq mm) statistics at different follow up period among the groups

Group		CECD (per sq mm)		
		Pre op	Post Operative 1 Month	Post Operative 3 Month
Group 1	N	40	40	40
	Mean	2489.48	2290.65	2248.78
	SD	244.64	245.08	243.06
	Median	2491.00	2298.00	2250.50
Group 2	N	40	40	40
	Mean	2586.95	2463.58	2432.35
	SD	268.68	269.55	271.90
	Median	2605.00	2480.50	2447.00
Total	N	80	80	80
	Mean	2538.21	2377.11	2340.56
	SD	259.98	270.36	272.39
	Median	2565.00	2395.50	2357.50
P Value LS		.094	.004	.002

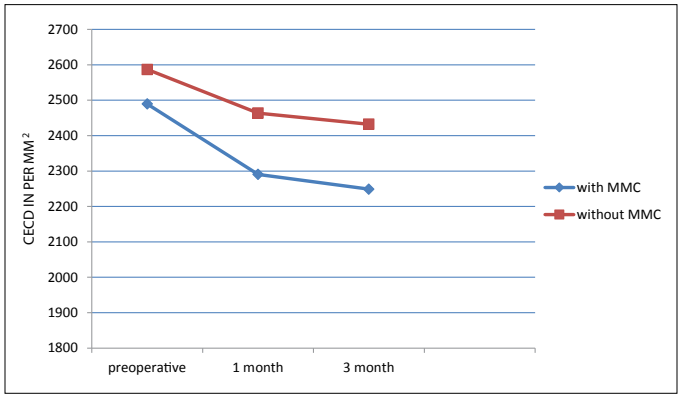

Figure 8: CECD (per sq mm) statistics at different follow up period among the groups

Table 3: CV% statistics at different follow up period among the groups

Group		CV%		
		Pre Op	1 m	3m
Group 1	N	40	40	40
	Mean	30.20	32.33	33.55
	SD	6.36	6.39	6.25
	Median	29.50	32.50	34.00
Group 2	N	40	40	40
	Mean	31.80	34.05	35.05
	SD	5.43	5.57	5.67
	Median	31.00	33.50	34.00
Total	N	80	80	80
	Mean	31.00	33.19	34.30
	SD	5.93	6.02	5.97
	Median	30.00	33.50	34.00
P Value LS		.230	.202	.264

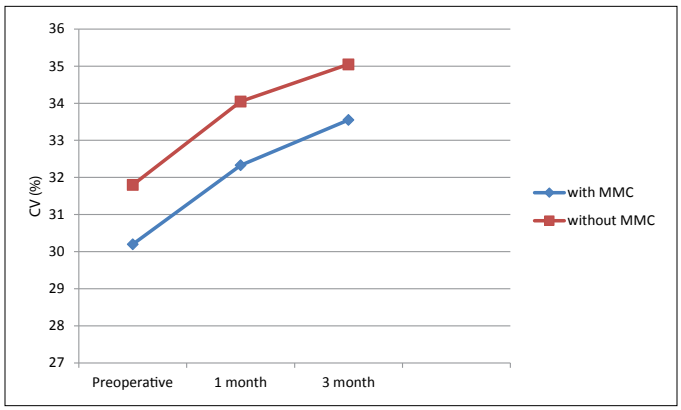

Figure 9: CV% statistics at different follow up period among the groups

Table 4: Hexagonality% statistics at different follow up period among the groups

Group		Hexagonality%		
		Pre op	Post Operative 1 Month	Post Operative 3 Month
Group 1	N	40	40	40
	Mean	54.23	51.70	50.40
	SD	4.59	4.65	4.71
	Median	54.00	51.50	50.50
Group 2	N	40	40	40
	Mean	53.03	51.38	50.43
	SD	4.33	4.40	4.33
	Median	53.00	51.50	51.00
Total	N	80	80	80
	Mean	53.63	51.54	50.41
	SD	4.47	4.50	4.49
	Median	53.50	51.50	51.00
P Value LS		.233	.749	.980

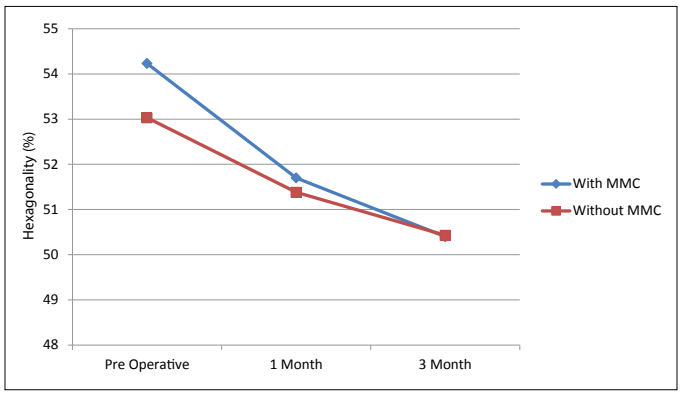

Figure 10: Hexagonality%statistics at different follow up period among the groups

Postoperatively, the mean CCT in group 1 increased to 529.88 ± 13.22 microns at 1 month and then reduced to 526.60 ± 12.97 microns at 3 months postoperatively. Similarly, in group 2 the mean CCT increased to 517.28 ± 14.09 microns at 1 month and then reduced to 514.78 ± 14.28 at 3 months postoperatively. Postoperatively the mean CCT increased initially and then reduced gradually at 3 months in both the groups. The difference between the two was staistically significant. (P=.000) (Table 5) (Figure 11)

Table 5: C.C.T (um) statistics at different follow up period among the groups

Group		C.C.T (um)		
		Pre op	Post Operative 1 Month	Post Operative 3 Month
Group 1	N	40	40	40
	Mean	515.75	529.88	526.60
	SD	13.445	13.22	12.97
	Median	515.00	529.50	527.00
Group 2	N	40	40	40
	Mean	513.08	517.28	514.78
	SD	14.161	14.09	14.28
	Median	514.50	518.50	516.50
Total	N	80	80	80
	Mean	514.41	523.58	520.69
	SD	13.786	14.99	14.80
	Median	515.00	523.00	520.50
P Value LS		0.38NS	.000	.000

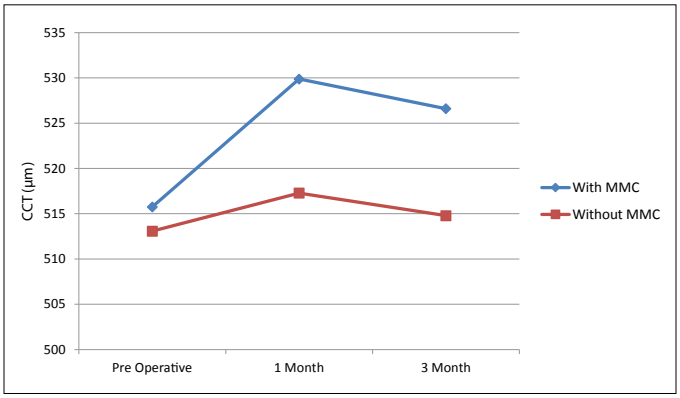

Figure 11: C.C.T (um) statistics at different follow up period among the groups

Discussion

The human corneal endothelium, which guarantees corneal transparency, is primarily a non-replicative tissue. In an immediate response to a loss of endothelial cells, the remaining cells enlarge and slide in an attempt to cover the posterior corneal surface fully, and this is reflected in a short term increase in the cell size and a decrease in the percentage of hexagonal cells. When the endothelium is stabilized after a period of rearrangement, the CV and the hexagonality shift toward the preoperative status.

There was no difference in endothelial cell count preoperatively in both groups.

We assessed the endothelial cell count, post-operatively at intervals of 1 month and 3 months and noted that, the mean endothelial cell loss was significantly higher in group 1 as compared to group 2 at each follow up. (P=0.004). The percentage of cell loss from baseline to postoperative month 1 in group 1 versus 2 was and 7.98% and 4.76%, respectively

(P = 0.004). CECD loss from baseline to postoperative month 3 in group I versus II was 9.66% and 5.97%, respectively (P = 0.002). CECD loss from months 1 to 3, in group I versus II was 1.68% and 1.21%, respectively.

This can be compared with the results published by Paulsen et al (2008)[8] reporting CECD loss of 9.6%, and 10.0% at postoperative months 3 and 12, respectively, following MMC augmented trabeculectomy with 0.2 mg/ml MMC. Similar study done by Zarie et al (2015)[9] reported 7.2% and 8.7% CECD loss at 1month and 3month, following MMC augmented trabeculectomy with 0.2 mg/ml MMC.

In our study, there was no statistical difference in the mean co-efficient of variance (CV) between the two groups in the pre-operative period. After the surgery the mean percentage of CV increased in both the groups. Though the mean percentage of CV gradually increased in both the groups, but it was not statistically significant. (P=0.264). Sano et al (1998)[10], reported a 1.9% increase in CV 2 to 3 weeks after MMC-augmented trabeculectomy, which was not statistically significant. Zarie et al (2015)[9] reported increase in CV after surgery in both study groups as compared to baseline. This change was not significant statistically (P>0.05).

In our study, there was no statistical difference in the hexagonality between the two groups in the pre-operative period. (P=0.233). After the surgery the mean percentage of hexagonality dropped in both the groups, but it was not statistically significant. Sano et al (2008)[10] reported decrease in hexagonality by 1.7% which was statistically not significant.

Talking of the central corneal thickness, the mean central corneal thickness (CCT) pre-operatively was slightly higher in group 1 than in group 2 but groups were comparable. (P=0.38). In our study, we observed that, postoperatively the mean CCT increased initially and then reduced gradually at 3 months in both the groups. The difference between the two was statistically significant. (P=.000)

This indicates that corneal swelling is probably the result of damage to endothelial cells by the surgery. Although endothelial cell density is a widely used parameter for the status of the cornea after surgery, it does not reflect the dynamics of the endothelial healing process that occurs in response to surgical trauma. The decrease in cell density reflects the surgical trauma itself, whereas the change in morphology is more closely associated with the process of repair.

To conclude, corneal endothelium is vulnerable to Mitomycin-C and these findings should be considered when planning trabeculectomy with Mitomycin-C as modest endothelial loss may result in corneal decompensation. Therefore, patient selection is very important.

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Address for correspondence

Shalini Dhiman MBBS, MS
Vill Chogan, PO Daroh Teh Palampur,
Distt Kangra, Himachal Pradesh,
India
Email id: shalinidhiman0408@gmail.com

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