Original articles

Effect of mitomycin C-aided trabeculectomy on conjunctival goblet cell density

Mukhopadhyay S[1], Thakur SKD[1], Dutta J[1], Prakash R[1], Shaw C[1], Gangopadhyay DN[2], Dutta H[1], Bhaduri G[1]
[1]Regional Institute of Ophthalmology, [2] Department of Dermatology, School of Tropical Medicine and Hygiene, Kolkata, India

Abstract

Introduction: Mitomycin C is gaining widespread popularity as an adjunctive with trabeculectomy, as it significantly increases the success rate of the procedure. But it is associated with serious sight-threatening complications.

Materials and methods: Twenty eyes planned for trabeculectomy from the glaucoma clinic were enrolled for the study after obtaining an informed consent. The baseline impression cytology was taken. Ten eyes underwent trabeculectomy with mitomycin C (Group A) and the rest underwent trabeculectomy without any antimetabolites (Group B). Impression cytology samples were taken on months 1, 3, 6, 9 and 12.

Results: In Group A, the difference between goblet cell density preoperatively and 12 month postoperatively was statistically significant ($p < 0.0001$). In Group B, the difference was not statistically significant. ($p = 0.27$).

Conclusion: Mitomycin C, though used to augment the success rate of trabeculectomy, has deleterious effect on the conjunctival goblet cell population as is evident from the conjunctival impression cytology.

Key words: trabeculectomy, mitomycin C, goblet cell, conjunctival impression cytology

Introduction

Trabeculectomy is the standard surgical procedure for lowering intraocular pressure in the treatment of glaucoma. The use of adjunctive antimetabolites like mitomycin C (MMC) has increased the success rates of trabeculectomy. But at the same time this has led to an increase in the frequency of thin avascular blebs and the consequences like delayed bleb leaks, hypotony, hypotonic maculopathy, blebitis and endophthalmitis. (Bindlish, 2002). The conjunctival surface needs to be evaluated in MMC aided trabeculectomy patients to understand the possible reasons for these adverse effects.

Conjunctival impression cytology (CIC) is a simple and painless method of obtaining specimens from the conjunctival surface to assess the morphology. It has been used to assay goblet cells in normal subjects and other ocular surface disorders.

The present study was conducted to evaluate changes in the conjunctival goblet cell density (GCD) in mitomycin C aided trabeculectomy by conjunctival impression cytology.

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Address of corresponding: Dr Sanjay Kumar Daulat Thakur Flat 1C, Rohini Apartment, 8 / 1A Canal Street, Behind Entally P.S., Kolkata 700 014
Phone: +91-98302 65740
Email: skdthakur@yahoo.com

Materials and methods

The study was done on 20 eyes of 20 patients with

primary open angle glaucoma or primary angle closure glaucoma who were scheduled for primary trabeculectomy with or without mitomycin C. The patients were selected from the Glaucoma Clinic at Regional Institute of Ophthalmology, Kolkata. Informed consent was obtained after the nature of the procedure had been fully explained to the subject. Baseline Conjunctival Impression cytology (CIC) was performed on the eyes of the patients.

They were divided into two equal groups. Group A (10 eyes) underwent primary trabeculectomy with single intraoperative exposure of mitomycin C (0.2mg/ml for 3 minutes). Group B (10 eyes) underwent primary trabeculectomy without the use of any adjunctive. This was followed by impression cytology postoperatively at the end of months 1, 3, 6, 9 and 12. All eyes with non functioning blebs or blebs with signs of inflammation or bleb leakage were excluded from the study. Patients with ocular surface disorders such as dry eye or history of surgery involving conjunctiva or contact lens wearers were excluded from the study. CIC samples were taken from the bulbar conjunctiva using standard protocol advocated by Tseng (1985). The cellulose acetate filter paper was cut into 5×7-mm strips. After instillation of one drop of topical anaesthetic to each eye, excessive tear fluids was wiped out and individual strips of the filter material were then pressed onto the nasal and temporal interpalpebral bulbar conjunctivae using a pair of smooth and flat ended forceps.

The filter paper was then gently smoothened onto the ocular surface by touching the forceps tip at each of the four corners of the paper against the ocular surface. The filter paper was removed by picking up the tip of the filter with the same forceps and following a peeling maneuver over the ocular surface. The filter paper was dropped into one of the sample bottles, which contained the fixative solution, containing glacial acetic acid 5 ml, 37% formaldehyde 5 ml and 70% ethyl alcohol 100 ml and the bottle was sealed by screw.

The specimen information was labeled accordingly, by entering the date of sample collection, patient's name (last, first and middle initials), medical record number, the eye involved, area of the conjunctiva from where the sample was removed. All the collected samples (preoperative and postoperative) were subjected to standard staining methods described by Tseng (1985) and goblet cell density was calculated and compared. The cells were counted at ×400 magnification. The mean total of ten such areas were recorded for each specimen. The mean nasal and temporal bulbar conjunctival densities for each eye were averaged and reported as goblet cell densities per high power field (field area=0.1885mm^2).

Statistics: The difference in the mean number of goblet cells was assessed through Student's t test. The significance level was considered at $p < 0.05$.

Results

The age of patients in Group A ranged from 38 to 67 years, while that of Group B was between 56 to 73 years. There were 6 males and 4 females in Group A, whereas in Group B there were 4 males and 6 females. Most of the patients (17 eyes) had primary open angle glaucoma (POAG), while the rest (3 eyes) had primary angle closure glaucoma (PACG). The goblet cell density (GCD) in Group A (mitomycin C aided trabeculectomy) preoperatively and postoperatively at months 1, 3, 6, 9 and12 is shown in table 1. The change in goblet cell density at 12 month postoperatively as compared to preoperative density was statistically significant by 2 tailed Student's paired t test ($p<0.0001$).

Table 1: Group A
Pre-operative and post-operative goblet cells density

No.	Pre Op GCD	Post op GCD				
		Month 1	*Month 3*	*Month 6*	*Month 9*	*Month 12*
1	184	182	180	172	160	159
2	180	178	175	165	160	155
3	190	190	184	170	167	164
4	210	202	195	184	175	178

No.	Pre Op GCD	Post op GCD				
		Month 1	*Month 3*	*Month 6*	*Month 9*	*Month 12*
5	197	190	187	180	174	175
6	175	170	165	159	149	146
7	190	180	170	163	158	156
8	180	174	172	155	152	150
9	205	196	195	191	185	181
10	198	190	188	180	174	170

GCD = Goblet cell density, Pre Op = Pre-operative, Post Op= Post-operative

Table 2: Group B
Pre-operative and post-operative goblet cells density

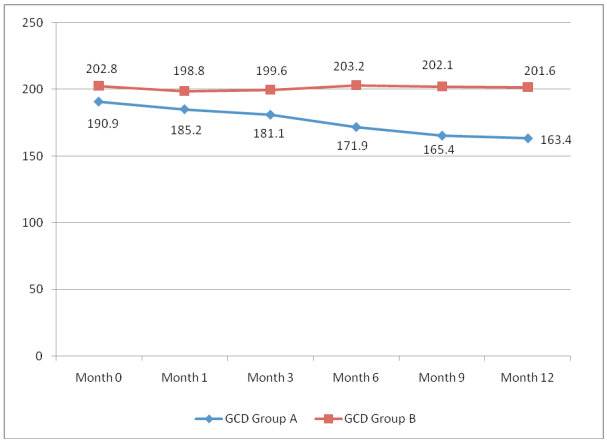
No.	Pre Op GCD	Post Op GCD				
		Month 1	*Month 3*	*Month 6*	*Month 9*	*Month 12*
1	190	191	189	188	190	192
2	192	191	190	192	190	193
3	185	182	180	185	185	187
4	200	197	198	199	199	204
5	198	196	194	200	199	195
6	210	203	209	210	207	208
7	220	215	219	220	215	218
8	230	222	225	230	232	225
9	205	201	200	208	204	199
10	198	190	192	200	200	195

GCD = Goblet cell density, Pre Op = Pre operative, Post Op= Post operative

The goblet cell density (GCD) in Group B (trabeculectomy alone) preoperatively and postoperatively at months 1, 3, 6, 9 and12 is shown in Table 2. The change in goblet cell density at 12 month postoperatively as compared to preoperative density was statistically not significant by 2 tailed Student's paired t test (p =0.27).

Figure 1 shows the changes in the mean goblet cell density over 12 month period in both the groups. The mean preoperative goblet cell density in Group A was 190.9 and the mean postoperative goblet cell density at 12 months was 163.4. In Group B the mean preoperative goblet cell density was 202.8 and the mean postoperative goblet cell density at 12 months was 201.6.

Figure 1: Graph showing changes in the mean goblet cell density over 12 month period in both the groups



It is interesting to note that in Group A the difference in goblet cell density preoperatively and at 1 month postoperatively was also statistically significant by 2 tailed Student's paired t test (p = 0.0004).

Discussion

Mitomycin C (MMC) was reported by Chen in 1983 to enhance the IOP lowering efficacy of trabeculectomy when applied intraoperatively in eyes at high risk of surgical failure. MMC is an antineoplastic antibiotic isolated from *Streptomyces caespitosus*. (Allingham et al, 2005). A retrospective study has shown that primary trabeculectomy with MMC maintained an IOP level of 15 mmHg or less in more than 80 % of patients after 1 year and in 60 % after 6 years suggesting that the use of MMC may be justified in primary trabeculectomies in patients with severe glaucoma (Beckers et al 2003). Chen et al (1987) and Palmer (1991) found overall success rates of 91 % and 84 % respectively after a single intraoperative administration of the MMC.

But the use of MMC is not without adverse effects. It is associated with many sight threatening complications. In a study by Bindlish et al on 123 eyes that underwent primary trabeculectomy with MMC, the mean preoperative and year 5 postop-

erative IOP were 25.79 and 9.91 mm Hg ($p<0.05$). Hypotony (IOP < 6) occurred in 42.2 % of eyes after a mean follow up of 26.1 months. Hypotony maculopathy occurred in 8.9 % of eyes at mean follow up of 33.7 months. Bleb leak occurred in 14.6 % of eyes at a mean follow up of 27.9 months. Blebitis occurred in 5.7 % of eyes at a mean follow up of 35.4 months and endophthalmitis occurred in 0.8 % of eyes at 15 months; 14.9 % of eyes lost 4 lines of visual acuity.

Anand et al (2006) studied the evolution of possible high risk features of conjunctival filtration blebs like avascularity, transconjunctival oozing (TCO) and leaks in 125 consecutive eyes of patients who had MMC enhanced glaucoma surgery. The mean time (95 % CI) for observing bleb avascularity, TCO and bleb leaks were 106 days, 208 days, and 609 days respectively. In eyes with avascular blebs, the probability of developing TCO and leaks was 77 % and 1 % at 6 months, 81 % and 12 % at 12 months, and 95 % and 26 % at 24 months respectively. Cox's regression analyses and log rank tests showed that eyes with larger avascular blebs (>4 mm) were more likely to develop TCO (hazard ratio 3.77, $p=0.001$).

Histologic studies in rabbits and monkeys have demonstrated that in MMC induced filtering blebs, there is a higher incidence of thin avascular bleb formation and acellular filtration sites with hypocellular bleb cavities (Khaw et al, 1993; Pasquale et al, 1992); these characteristics have undoubtedly contributed to the significant prevalence of leakage and ocular hypotony.

Liang et al (2009) conducted histopathologic examination of a normally functioning mitomycin C trabeculectomy site obtained from an eye enucleated for iris melanoma. They observed that the conjunctival histopathology is significantly altered. The conjunctival stroma consisted of loose connective tissue, traversed by capillaries and scattered small cystic spaces lined by endothelial cells. There were no goblet cells and few inflammatory cells and fibroblasts.

Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium. These cells can then be subjected to histological analysis. Applications for impression cytology include diagnosing a wide range of ocular surface disorders, documenting sequential changes in the conjunctival and corneal surface over time and monitoring effects of treatment. It is non invasive, relatively easy to perform and yields reliable information about the area sampled with minimal discomfort to the patient. (Singh et al, 2005)

Kim used impression cytology to evaluate the conjunctival surface change after filtering surgery and its association with mitomycin C. A total of 22 eyes of 19 patients were included in the study and the mean duration after trabeculectomy was 20.41+/-14.46 months. During trabeculectomy, adjunctive MMC was used in six eyes. Abnormal impressions, demonstrated as disturbed epithelial and goblet cell morphology and a decrease in the numbers of both cells were found in 9 eyes (40.9 %). With regard to prevalence of abnormal impressions, there was statistically significant differences between the use or non use of mitomycin C ($p=0.013$). This study revealed that filtering surgery causes long term damage to the conjunctival epithelium overlying a filtering bleb, especially in a patient with a thin cystic bleb or one has been treated with mitomycin C.

In our study there was a statistically significant decrease in goblet cell density at 12 months postoperatively compared to preoperative density in mitomycin C aided trabeculectomy ($p<0.0001$). There was no such statistically significant difference in the control group in which primary trabeculectomy was done without mitomycin C. There was a significant drop in the goblet cell density even 1 month postoperatively in MMC aided trabeculectomy group.

Thin cystic blebs, enhanced by MMC increase the risk of conjunctival surface breakdown, with ooze or aqueous leaks; bleb related infection may indicate a compromised conjunctival barrier. Fragile

blebs may subsequently be ruptured by even minimal trauma, increasing their vulnerability to bacterial invasion (Kim, 1997).

Although mitomycin C increases the success rates of trabeculectomy, it has serious adverse effects on the conjunctival tissue. It has already been proven by earlier studies that MMC enhanced trabeculectomy results in thin cystic or avascular blebs which predispose them to infections.

Conclusion

The goblet cell density significantly reduces in patients exposed to mitomycin C during trabeculectomy which may predispose these eyes to adverse effects.

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