Original Article

Simultaneous use of amniotic membrane and Mitomycin C in trabeculectomy for primary glaucoma

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Purpose: This study aimed to propose the role of amniotic membrane transplantation (AMT) as an additional modulator in primary Mitomycin C (MMC)-augmented trabeculectomy. **Methods:** This was a randomized prospective interventional study. Forty eyes of 39 adult patients with uncontrolled primary glaucoma were randomly divided into two equal groups. Control group underwent trabeculectomy augmented with MMC while the study group underwent additional AMT. Patients were followed up for 12 months and outcomes measured were intraocular pressure (IOP), need for additional intervention, and bleb morphology. **Results:** Complete success (defined as IOP <16 mmHg on no medication) could be achieved in 85% eyes in study group while it was 60% in control group ($P = 0.04$). IOP reduced by 71.1% in study group from 41.9 ± 10.6 to 12.1 ± 2.7 mmHg and from 40.5 ± 8.5 to 12.8 ± 4.5 mmHg in control group, a decline of 68.29%. Blebs in AMT group showed better bleb morphology in terms of significantly better extent (E3) on day 1 ($P = 0.03$) and better height (H2 and H3) ($P = 0.04$), according to the Indiana Bleb Appearance Grading Scale, at all follow-up visits along with normal vascularity. The study group required significantly lesser ($P = 0.03$) bleb needlings as compared to control group. **Conclusion:** Amnion enhanced the efficacy of MMC-modulated trabeculectomy in terms of eyes with complete success and lesser interventions such as bleb needling. This reiterates the role of amnion as a safe and effective bleb modulator. A diffusely elevated bleb with healthier conjunctiva can go a long way in predicting better health and longevity of the bleb.

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Success of trabeculectomy is linked with interruption of the physiological wound-healing process. It is pertinent to maintain the patency of this newly created fistula; the major cause of failure being fibroblast proliferation causing subconjunctival and episcleral fibrosis. Introduction of anti-fibrotics such as Mitomycin C (MMC) and 5-flourouracil (5-FU) has revolutionized the modern-day glaucoma surgery, especially in cases at high risk of failure.[1-4] Many authors have reported remarkable effects of MMC both in primary and various refractory glaucomas.[1-3] However, the associated complications cannot be overlooked. Through a sustained cytotoxic effect on vascular endothelial cells and fibroblasts, MMC predisposes to bleb avascularity and transconjunctival oozing, ultimately leading to shallow anterior chamber (AC), endothelial cell loss (10%–11% more), cataract, hyphema, hypotony (1.3%–33%), hypotonous maculopathy (1%–14%), choroidal effusion, and late-onset blebitis and bleb-related endophthalmitis,[1-3] hence the need for more specific inhibitors of wound-healing pathway. The quest for finding a more physiological material as a bleb modulator while reducing the complication rate has led to the consideration of amniotic membrane. Easy availability, nonimmunogenic, anti-microbial, anti-protease, and anti-inflammatory activities are some of the properties which support its use in eyes undergoing trabeculectomy.[5] Used for the 1st time with MMC in 1988, Fujishima *et al*. reported a successful outcome (intraocular pressure [IOP] <20 mmHg) in 13 out of 14 glaucomatous eyes.[6] Acting at different levels in the wound-healing pathway, theoretically, amnion may be additive to MMC in achieving better IOP control and a stable bleb vitality and efficacy. This study was conducted to assess the role of amniotic membrane as an additional modulator in primary MMC-augmented trabeculectomy.

Methods

A prospective, randomized interventional study was conducted at the glaucoma services of a tertiary eye hospital and research center. The study was conducted after obtaining prior Institutional Ethical Committee approval and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all the patients. A total of 40 eyes of 39 patients were studied. Patients of primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG) fulfilling the following criteria were included:

1. IOP >21 mmHg with at least two topical antiglaucoma medications
2. Patients willing for 6-month follow-up and investigations
3. Age >35 years.

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Patients with any form of secondary glaucoma (uveitic, neovascular, lenticular, pigmentary, and pseudo-exfoliation glaucoma), a history of prior surgical interventions in the form of past glaucoma filtration surgery, cataract surgery, or any other surgery involving conjunctiva, coexisting conjunctival disease, any history of systemic illness, or use of anticoagulants were excluded. An inability to control IOP (<21 mmHg) with at least two topical antiglaucoma medications in POAG and/or inability to relieve pupillary block (by prior laser iridotomy) due to more than 180° posterior synechiae or synechial angle closure in cases with PACG were indications for surgery. The participants were randomly divided into two equal groups. Study group (Group A) underwent trabeculectomy augmented intraoperatively with both MMC and amniotic membrane transplant (AMT) whereas control group (Group B) underwent trabeculectomy augmented intraoperatively with MMC only. A complete glaucoma workup was done for all patients preoperatively including best-corrected visual acuity (BCVA), applanation tonometry, gonioscopy, slit-lamp examination, fundoscopy with 90 D, and visual fields, where possible.

Procedure

A single surgeon operated on all patients. The procedure was carried out under full aseptic conditions. Peribulbar anesthesia (8 ml) was given with lignocaine and bupivacaine in a proportion of 6:4. Globe traction was achieved by a superior rectus bridle suture. Fornix-based conjunctival flap was raised and bleeders were cauterized with light bipolar wet-field cautery. 0.2 mg/ml MMC-soaked Merocel® sponges were applied subconjunctivally for 2 min followed by a thorough wash with 20 ml of 20% ringer lactate. Partial-thickness limbus-based rectangular scleral flap sized 4 mm × 3.5 mm was raised up to the edge of clear cornea. A paracentesis port was placed outside of the flap area using a lancet. Intracameral pilocarpine (2%) was injected through the paracentesis port. AC was then entered with part entry of 3.2 mm keratome at limbo-corneal junction and re-entered 1 mm behind, directing it in the iris plane all the times, taking full care that AC was not lost any time. The two incisions were joined with Vannas scissors, trabecular block (3.0 mm × 1.0 mm) was excised, and a peripheral iridectomy was done. Scleral flap was sutured with three or four 10-0 nylon sutures. A single releasable suture was applied in both groups with 10-0 nylon. The bleb was titrated for optimum percolation. In Group A, amnion was applied over the scleral flap with the mesenchymal (sticky) surface in contact with the sclera and anchored at two peri-limbal sites with 10-0 nylon sutures. (The amnion was obtained under sterile conditions after elective cesarean section from a previously tested sero-negative donor, processed and preserved at −70°C–80°C. After undergoing sterilization, it was stored and transported under stringent conditions in a similarly cooled packaging for ocular application). The conjunctiva was then closed with two wing sutures at the limbus using 8-0 nylon. Bleb-forming sutures were applied using 10-0 nylon to achieve a watertight closure. Subconjunctival injection of gentamicin + dexamethasone (0.5 ml + 0.5 ml) was given in the inferior fornix in all patients. Postoperative topical corticosteroids (prednisolone acetate), cyclopentolate 1%, and antibiotic drops were given for a maximum duration of 6 weeks and superficial sutures were removed thereafter; a minimum of 6 weeks. Releasable suture was released within 7–10 days in all cases.

Postoperative follow-up and outcome assessment

All patients were followed up for a minimum period of 1 year (day 1, 1 week, 3 weeks, 2, 4, 6, 8, 10, and 12 months with ±15 days for last three visits). The outcome was assessed on the basis of IOP achieved, complications, need for intervention in the form of antiglaucoma medications and/or 5-FU bleb needling, and bleb morphology. Complete success was defined as IOP 6–16 mmHg without any antiglaucoma medication at 1 year and qualified success as IOP ≤16 mmHg after intervention in the form of one antiglaucoma medication or supplemental 5-FU needling. Failure was noted in cases where IOP remained >16 mmHg, <6 mmHg, and/or need for additional surgical intervention such as AC reformation, cataract surgery, repeat trabeculectomy, or need for two or more antiglaucoma medications even after bleb needling.

The bleb assessment was done using slit-lamp images which were then compared with the standard images published as the Indiana Bleb Appearance Grading Scale (IBAGS).[7] Any statistical difference in the bleb morphology parameters between the two groups was compared on the first postoperative day, 6 months, and 12 months.

Statistical evaluation

The data were analyzed by SPSS software, version 16 (SPSS, Inc., Chicago, IL, USA). Qualitative data were analyzed by Fisher's exact test or Chi-square test. Quantitative data were analyzed by Mann–Whitney test for comparison between the groups and Wilcoxon signed-rank test for pre- and post-operative comparisons in the same group. $P < 0.05$ was considered statistically significant.

Results

The age of the patients ranged from 35 to 70 years. All eyes were phakic and media clear enough to visualize the fundus. A dominance of angle-closure patients was noted in both the groups with only three patients being open-angle cases. The two groups matched with regard to demographic profile and baseline characteristics [Table 1].

Mean BCVA in both groups remained stable at 12-month follow-up, i.e., from 0.75 ± 0.64 to 0.80 ± 0.63 logarithm of the minimum angle of resolution (logMAR) in study group and

Table 1: Baseline and demographic characteristics of both groups

	Group A	Group B	*P*
Age	50.95±9.54*	54.65±11.05*	0.13
Gender			
Male	9 (20)	11 (20)	0.26
Female	11 (20)	9 (20)	
Diagnosis			
PACG	18 (20)	19 (20)	0.27
POAG	2 (20)	1 (20)	
BCVA (logMAR)	0.75±0.64*	0.56±0.34*	0.12
IOP (mmHg)	41.86±10.59*	40.52±8.46*	0.33

*Mean±SD. PACG: Primary angle-closure glaucoma, POAG: Primary open-angle glaucoma, BCVA: Best-corrected visual acuity, IOP: Intraocular pressure, SD: Standard deviation, LogMAR: Logarithm of the minimum angle of resolution

from 0.56 ± 0.34 to 0.60 ± 0.36 logMAR in control group ($P = 0.13$ and 0.18 in Groups A and B, respectively).

In Group A (study group), mean preoperative IOP decreased by 71.09% from 41.9 ± 10.6 mmHg to mean postoperative IOP of 12.1 ± 2.7 mmHg at 1 year, while in Group B (control), a decline of 68.29% was noted from 40.5 ± 8.5 to 12.8 ± 4.5 mmHg [Fig. 1]. While both the groups were comparable in terms of postoperative IOP control at all follow-up visits ($P > 0.05$), the study group reported significantly better rate of complete success of 85% (17/20 eyes) vis-à-vis 60% (12/20 eyes) in control group ($P = 0.04$) at 12 months. Rest of the three eyes in study group had a qualified control while none reported failure. Qualified control was seen in 35% (7/20) of eyes in control Group B, while one patient categorized as failure and required re-surgery [Table 2]. Hence, significantly more patients in control group (7/20) required intervention in the form of 5-FU needling and one antiglaucoma medication in comparison to study group ($P = 0.03$) to maintain IOP <16 mmHg. Furthermore, 5-FU needling was required earlier, at around 1 month, in two eyes in control group; while the rest needed intervention between 3 and 6 months. The number of 5-FU needlings required in study group was significantly lesser, i.e., two as compared to seven in control group, $P = 0.03$ [Table 3].

The surgery was uneventful in all patients. None of the patients in either group developed complications such as hypotonous maculopathy, vision snuff out, suprachoroidal hemorrhage, or endophthalmitis. Nonspecific early postoperative complications in both groups are summarized in Table 3. One patient in control group showed persistent postoperative hypotony and developed choroidal detachment around 1 month postsurgery which resolved on oral steroids and cycloplegic while one patient in study group showed blebitis, requiring topical antibiotics, namely, moxifloxacin and fortified tobramycin two hourly.

The bleb morphology was studied according to the IBAGS,[7] as mentioned above. On day 1, Group A had statistically more number (20/20 eyes) of higher blebs (H2+H3) ($P = 0.00$) persisting until the last follow-up ($P = 0.04$) by when 80% (16/20 eyes) settled in H2 + H3 category and only 20% (4/20 eyes) had low blebs in comparison to 55% (11/20 eyes) with H2 + H3 blebs and 40% low blebs in control group [Figs. 2-4]. In addition, there was a statistically significant difference between the extent of blebs in the two groups, with Group A having 18/20 (90%) blebs in E3 category as against 13/20 (65%) in Group B ($P = 0.03$) on day 1. However, this difference in bleb extent gradually decreased and by 12 months 70% (14/20) in Group A and 65% (13/20) in Group B settled in E2 category ($P = 0.37$) [Fig. 5]. In terms of vascularity, there was a gradual decline from moderate vascularity (V3) on day 1 and by 1 year all patients had V1 blebs, i.e., avascular polycystic except one eye in control group which showed a scarred bleb and was reported as failure [Fig. 6]. In either group, there were no cases of bleb leak anytime.

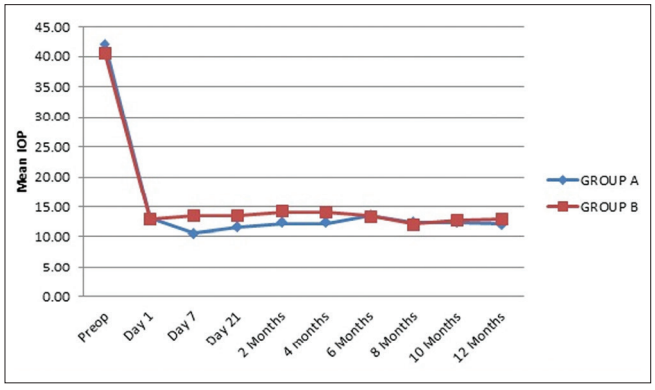

Figure 1: Mean intraocular pressure at follow-up visits

Discussion

Antimetabolite agents as modulators of fibrotic process have been in use for over two decades, especially in high-risk cases. However, these antimetabolites often act as a double-edged sword resulting in complications such as hypotonous maculopathy and endophthalmitis.[1-3] Of late, a lot of interest

Table 2: Number of eyes with complete success, qualified success, and failure

Success	Frequency (%)		*P*
	Group A	Group B	
Complete success	17 (85)	12 (60)	0.04
Qualified success	3 (15)	7 (35)	0.07
Failure	0	1 (5)	0.15
Total	20 (100)	20 (100)	

Table 3: Complications and interventions

Complications and interventions	Group A	Group B
Streak hyphema	5% (1/20)	15% (3/20)
Streak hypopyon	5% (1/20)	5% (1/20)
Blebitis	5% (1/20)	None
Choroidal detachment	None	5% (1/20)
Persistent epithelial defect	None	5% (1/20)
Superior rectus hematoma	None	5% (1/20)
Cataract progression	15% (3/20)	40% (8/20)
5-FU needling	10% (2/20)	35% (7/20)
Phacoemulsification and planned for re-trabeculectomy	None	5% (1/20)

5-FU: 5-flourouracil

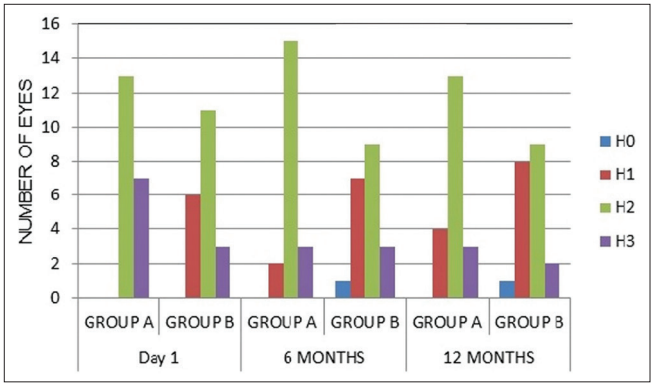

Figure 2: Height of blebs in Groups A and B postoperatively at day 1, 6 months, and 12 months. H0: Flat bleb without visible elevation, H1: Low bleb elevation, H2: Moderate bleb elevation, H3: High bleb as compared to standard images

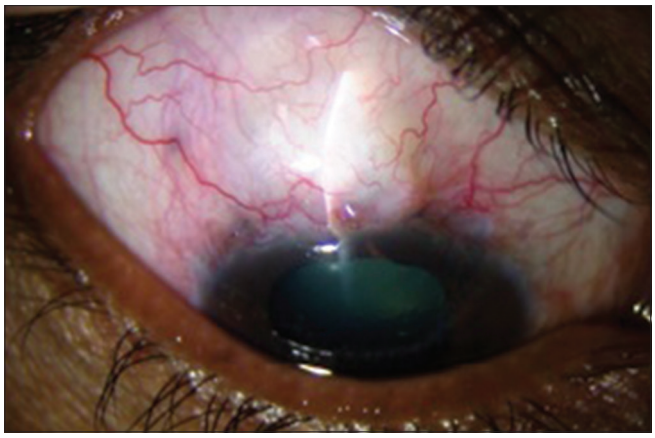

Figure 3: Three-month postoperative photograph of a patient in Group A showing a bleb with normal vasculature, diffuse bleb, and glistening conjunctiva

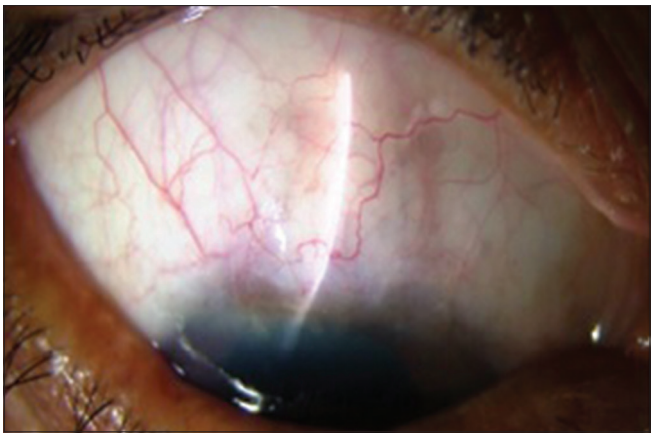

Figure 4: Three-month postoperative photograph of a patient in Group B showing a pale bleb

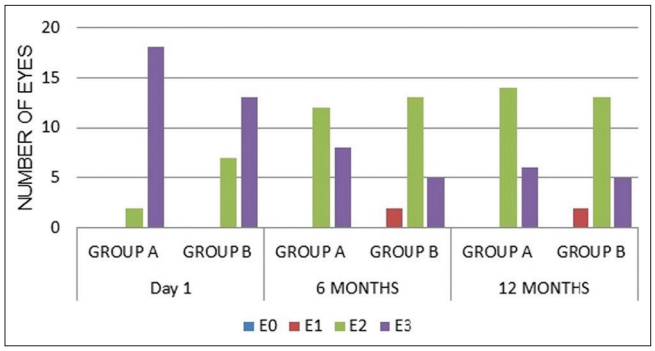

Figure 5: Extent of blebs in Groups A and B postoperatively at day 1, 6 months, and 12 months. E0: No visible bleb extent or <1 clock hour, E1: Extent ≥1 clock hour but <2 clock hours, E2: Between 2 and 4 clock hours, E3: Extent ≥4 clock hours

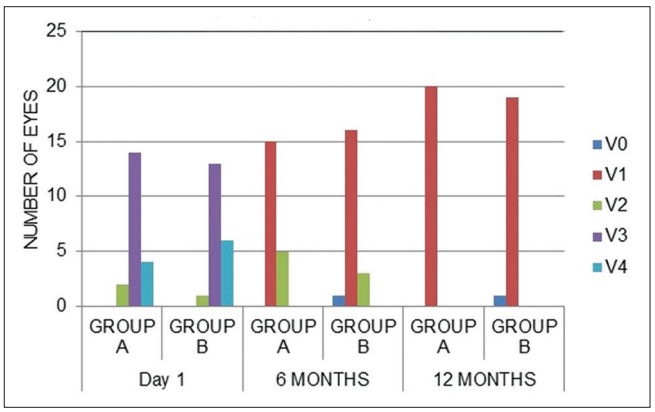

Figure 6: Vascularity of blebs in Groups A and B postoperatively at day 1, 6 months, and 12 months. V0: Avascular/white (no microcysts visible on slit-lamp examination), V1: Avascular/polycystic (microcysts of the conjunctiva visible on slit-lamp examination), V2: Mild vascularity, V3: Moderate vascularity, V4: Extensive vascularity (vascular engorgement)

is emerging in assessing the role of human amniotic membrane in reducing these complications. It supposedly forms an anatomical barrier keeping potentially adhesive surfaces apart and downregulates transforming growth factor-β2, the main isoform in aqueous involved in scarring.[5] Furthermore, it effectively suppresses fibroblastic and macrophage responses and its avascular stroma inhibits incursion of new vessels.[8] Initial reports showed favorable results in POAG eyes undergoing primary trabeculectomy.[9] Subsequently, amnion was reported to be efficacious in refractory glaucoma surgery with 80%–95% complete success when used alone,[10] or in conjunction with MMC.[10,11] Our study shows comparable results with complete success observed in 85% eyes when amnion augmentation was done in comparison to 60% with MMC alone at the end of 1 year. This increased to 100% and 95% in study and control groups, respectively, with the use of one antiglaucoma medication and/or 5-FU-needling. There was a significant fall in mean IOP postoperatively in both groups, being maintained throughout the study period. Although the IOP reduction was comparable, MMC-only eyes needed significantly more number of interventions to achieve the same. Better survival curve and lesser risk of developing IOP ≥21 mmHg and subsequent failure along with more diffuse and mildly vascularized blebs in the AMT group have been similarly reported previously in POAG cases.[12] Our study emphasizes the same in PACG eyes. The surgical techniques reported previously have mostly placed the amnion under the scleral flap,[6,9] both under and around,[11] or sutured as a double layer, both under and over the scleral flap after impregnating with MMC.[10] Our study reports favorable results with subconjunctival placement of single-layer amnion, which saves surgical time as compared to the above-mentioned techniques.

In terms of bleb morphology, IBAGS and all other previous classifications have described an ideal bleb as diffuse, moderately high, and avascular polycyst corresponding to a score range of H2–3, E2–3, V1, and S0. In our study, Group A showed significantly more number of higher (H2+H3) blebs from the beginning which persisted even at the end of 1 year. Thus, this indicates that adding amnion with MMC increases the chances of forming a diffusely elevated bleb with a much healthier overlying conjunctiva. Although these results can very well be attributed to the conjunctival bulge due to the physical bulk of amniotic membrane in the initial follow-up visits, there was a notable persistence of good bleb morphology and healthier conjunctiva along with lower requirement of secondary interventions such as 5-FU needling later in the

study. This suggests its possible protective role in saving the already doubly compromised conjunctiva due to surgical and MMC insult, from any additional surgical insult. It seems that amnion provides a scaffold for the growth and repair of acutely injured conjunctiva over it and prevents the conjunctiva from thinning and becoming leaky.

Conclusion

Thus, AMT seemed to preserve the integrity of the overlying conjunctiva keeping the bleb in a more physiological state which goes a long way in predicting better health of the bleb in the long term. However, the bleb is in a state of continuous remodeling even many years after surgery and requires adequate time postoperatively for consolidation of its morphological characteristics. The occurrence of failures in the late postoperative period being a well-known fact, results of this study cannot be extrapolated and comparisons are better made after more years of follow-up. Hence, it is recommended that more longitudinal studies with larger sample size and longer observation period must be undertaken to accurately elucidate the effectiveness of AMT as a cost-effective bleb modulator, demanding no extra learning curve as against modern patented surgical devices used in trabeculectomy.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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