Simultaneous Use of Amniotic Membrane and Mitomycin C in Trabeculectomy for Primary Glaucoma: A Pilot Study

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Abstract

Purpose: Role of amniotic membrane transplantation as an additional modulator in primary Mitomycin C augmented trabeculectomy.

Design: Randomised prospective interventional study.

Methods: 40 eyes of 39 adult patients with uncontrolled primary glaucoma were randomly divided into 2 equal groups. Control group underwent trabeculectomy augmented with Mitomycin C while the study group underwent additional amniotic membrane transplantation. Patients were followed up for 12 months and outcomes measured were Intraocular Pressure, need for additional intervention and bleb morphology.

Results: Complete success (defined as intraocular pressure <16 mmHg on no medication) could be achieved in 85% eyes in study group while it was 60% in control group (p=0.04). Intraocular pressure reduced by 71.1% in study group from 41.9 ± 10.6 mmHg to 12.1±2.7 mm Hg and from 40.5 ± 8.5 mmHg to 12.8 ± 4.5 mmHg in control group, a decline of 68.29%.

Blebs in amniotic membrane transplantation 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 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 Mitomycin C modulated trabeculectomy in terms of eyes with complete success and lesser interventions like 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.

Keywords: Trabeculectomy; Antifibrotics; Mitomycin C; Amniotic membrane

Introduction

Success of trabeculectomy is linked to 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 sub-conjunctival and episcleral fibrosis. Introduction of anti-fibrotics like Mitomycin C (MMC) and 5-Flourouracil (5-FU) has revolutionized 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 trans-conjunctival oozing ultimately leading to shallow anterior chamber, cataract, hyphema, hypotony, hypotonous maculopathy, 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, non-immunogenic, 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, reported a successful outcome (IOP<20 mm Hg) 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 intraocular pressure (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.

Material and Methods

A prospective, randomized interventional study was conducted at the glaucoma services of a tertiary eye hospital and research centre. The study was conducted after obtaining prior institutional ethical committee approval & it adhered to the tenets of the Declaration of Helsinki. Informed consent was taken from all the patients. 40 eyes of 39 patients were enrolled. Patients of Primary Open Angle Glaucoma...
and Primary Angle Closure Glaucoma fulfilling the following criteria were included;

- IOP >21 mm of Hg with at least two topical anti glaucoma medication.
- Patient willing for 6month follow-up and investigations.
- Age>35 years.

Patients with any form of secondary glaucoma (uveitic, neovascular, lenticular, pigmentary and pseudo-exfoliation glaucoma), history of prior surgical interventions in the form of past glaucoma filtration surgery, cataract surgery, or any other surgery involving conjunctiva, co-existing 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 anti glaucoma medications in primary open angle glaucoma and/or inability to relieve pupillary block (by prior laser iridotomy) in cases with primary angle closure glaucoma (PACG) were indications for surgery. The subjects were randomly divided into two equal groups. Study group (Group A) underwent trabeculectomy augmented intraoperatively with both Mitomycin C (MMC) and Amniotic Membrane Transplant (AMT) whereas control group (Group B) underwent trabeculectomy augmented intraoperatively with MMC only. A complete glaucoma work up was done for all patients preoperatively including best corrected visual acuity (BCVA), applanation tonometry, gonioscopy, slit lamp examination, fundoscopy with 90D and visual fields, where possible.

Procedure

A single surgeon operated on all patients. The procedure was carried out under full aseptic conditions. Peribulbar anaesthesia (8 ml) was given with lignocaine and bupivicaine in a proportion of 6:4. Globe traction was achieved by a superior rectus bridle suture. Fornix based conjunctival flap was raised and bleeders cauterized with light bipolar wet field cautery. 0.2 mg/ml MMC soaked merocel sponges were applied subconjunctivally for 2 minutes followed by a thorough wash with 20 ml of 20% Ringer Lactate. Partial thickness limbus based rectangular scleral flap sized 4 × 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. Anterior chamber 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 time, taking full care that anterior chamber was not lost any time. The two incisions were joined with Vannas scissors, trabecular block (3.0 × 1.0 mm2) application.) Conjunctiva was then closed with two wing sutures at the fornix in all subjects. 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.

Post-operative follow up & 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 intraocular pressure achieved, complications, need for intervention in form of anti-glaucoma medications and/or 5 FU bleb needling and bleb morphology. Complete success was defined as IOP 6-16 mmHg without any anti-glaucoma medication at 1 year and qualified success as IOP ≤ 16 mmHg after intervention in the form of one anti glaucoma 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 like AC reformation, cataract surgery, repeat trabeculectomy or need for two or more anti-glaucoma medications even after bleb needling.

The bleb assessment was done using slit lamp images which were then compared with the standard images published as Indiana Bleb Appearance Grading Scale (IBAGS) [7]. Any statistical difference in the bleb morphology parameters between the 2 groups was compared on the 1st postoperative day, 6 months and 12 months.

Statistical evaluation

The data was analysed by SPSS software, version 16 (SPSS, Inc., Chicago, IL). Qualitative data was analysed by Fisher Exact test or Chi-square test. Quantitative data was analysed by Mann-Whitney test for comparison between the 2 groups and Wilcoxon Sign Rank test for pre and postoperative comparison in the same group. P value of <0.05 was considered significant.

Results

The age of subjects ranged from 35-70 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 regards to demographic profile and baseline characteristics (Table 1).

| Age | Gender | Diagnosis | BCVA (logMAR) | IOP (mmHg) |
|-----|--------|-----------|---------------|------------|
| 50.95 ± 9.54* | Male | PACG | 0.75 ± 0.64* | 41.86 ± 10.59* |
| 54.65 ± 11.05* | Female | POAG | 0.56 ± 0.34* | 40.52 ± 8.46* |

*Mean ± standard deviation; PACG: Primary Angle Closure Glaucoma; POAG: Primary Open Angle Glaucoma; BCVA: Best Corrected Visual Acuity; IOP: Intraocular Pressure

Table 1: Baseline and demographic characteristics of both groups

Mean BCVA in both groups remained stable at 12 month follow-up i.e. from 0.75 ± 0.64 logMAR to 0.80 ± 0.63 logMAR in study group.
and from 0.56 ± 0.34 logMAR to 0.60 ± 0.36 logMAR in control group (p=0.13 & 0.18 group A & B respectively).

In the study group (A), mean pre-operative IOP decreased by 71.09% from 41.9 ± 10.6 mm Hg to mean postoperative IOP of 12.1 ± 2.7 mmHg at 1 year while in control group (B) a decline of 68.29% was noted from 40.5 ± 8.5 mmHg to 12.8 ± 4.5 mm Hg (Figure 1). While both the groups were comparable in terms of post-operative 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 three eyes in study group had a qualified control while none reported failure. Qualified control was seen in 35% (7/20) 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 form of 5-FU needling and one anti-glaucoma medication in comparison to study group (p=0.03) in order to maintain IOP <16 mmHg. Also 5-FU needling was required earlier, at around 1 month, in two eyes in control group; while rest needed intervention between 3-6 months. Number of 5-FU needlings required in study group were significantly lesser i.e. two as compared to seven in control group, p=0.03 (Table 3).

The bleb morphology was studied according to IBAGS, 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 (Figures 2-4). Also 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) (Figure 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 (Figure 6). In either group, there were no cases of bleb leak anytime.

Table 3: Complications and Interventions.

| Complications & Interventions | Group A | Group B |
|-------------------------------|---------|---------|
| Streak hypopyon               | 5% (1/20) | 15% (3/20) |
| Blebitis                      | 5% (1/20) | 5% (1/20) |
| 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) |

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

| Success                         | Group A | Group B | P-value |
|---------------------------------|---------|---------|---------|
| Frequency                       | 17      | 12      | 60      | 0.04    |
| %                               | 85      | 76      | 60      |         |
| Complete Success                | 17      | 12      | 60      | 0.04    |
| Qualified Success               | 3       | 7       | 35      | 0.07    |
| Failure                         | 0       | 1       | 5       | 0.15    |
| Total                           | 20      | 20      | 100     |         |

Figure 1: Mean Intraocular Pressure at follow-up visits.

Figure 2: Height of blebs in groups A and B postoperatively at Day1, 6 & 12 Months. H0: Flat bleb without visible elevation; H1: Low bleb elevation; H2: Moderate bleb elevation; H3: High bleb as compared to standard images.

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

The surgery was uneventful in all patients. None of the patients in either group developed complications like hypotonous maculopathy, vision snuff out, supra-choroidal haemorrhage or endophthalmitis. Non-specific early post-operative complications in both groups are given in Table 3. One patient in control group showed persistent post-op hypotony and developed choroidal detachment around 1 month post-surgery which resolved on oral steroids and cycloplegic while one patient in study group showed blebitis, requiring topical antibiotics viz. moxifloxacin and fortified tobramycin 2 hourly.

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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 like hypotonous maculopathy and endophthalmitis [1-3]. Of late, a lot of interest 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 down regulates transforming growth factor (TGF-β2), the main isoform in aqueous involved in scarring [5]. Also it effectively suppresses fibroblastic and macrophage response and its avascular stroma inhibits incursion of new vessels [8]. Initial reports showed favourable 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 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 & control group respectively with the use of one anti glaucoma medication and/or 5FU-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 vascularised blebs in the AMT group have been similarly reported previously in POAG cases [12]. Our study emphasizes the same in PACG eyes. The surgical technique 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 favourable results with sub-conjunctival placement of single layer amnion, which saves surgical time as compared to above techniques.

In terms of bleb morphology, IBAGS and all other previous classifications have described an ideal bleb as diffuse, moderately high...
and avascular polycystic corresponding to a score range of H2-3, E2-3, V1, 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 like 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.

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 remodelling even many years after surgery and requires adequate time post-operatively for consolidation of its morphological characters. 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 Amniotic Membrane Transplantation as a cost effective bleb modulator demanding no extra learning curve as against modern patented surgical devices used in trabeculectomy.

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