Subconjunctival bevacizumab versus mitomycin c as adjuvant treatment to subscleral trabeculectomy

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Aim To compare the clinical outcome of adjuvant subconjunctival bevacizumab (BVZ) injection versus mitomycin C (MMC) during subscleral trabeculectomy.

Patients and methods In this study, 20 patients who were divided into two groups: group A included 10 (20 eyes) patients who underwent subscleral trabeculectomy with subconjunctival injection of 1.25 mg/0.1 ml BVZ, and group B included 10 (20 eyes) patients who underwent subscleral trabeculectomy with adjuvant intraoperative use of 0.2 mg/ml MMC for 2 min. This prospective nonrandomized clinical comparative study was conducted in Nour-El-Hayaha Eye Center (Cairo). Patients were diagnosed for primary open angle glaucoma with uncontrolled intraocular tension by maximum tolerable antiglaucoma therapy. The study excluded patients with neovascular glaucoma, congenital and juvenile glaucoma, history of ocular pathology, or surgery as retinal surgeries and uveitis.

Results Twenty patients were considered for the study, nine (45%) patients (18 eyes) were males and 11 (55%) patients (22 eyes) were females. The patients' ages ranged from 35 to 65 years (mean age 55.2±8.3). Mild hyphema, in one eye in group A (8.33%), wound leak in one (8.33%) eye in each group, and shallow anterior chamber in two (16.7%) cases in each group. It was the most common encountered complication in our study. One (8.33%) case of shallow anterior chamber in group A led to choroidal effusion. One (8.33%) case in group B developed late bleb-related endophthalmitis after 3 months, which ended in phthisis bulbi.

Conclusion The success of glaucoma filtration surgery is heralded by a wound healing response mainly mediated by fibroblast proliferation, migration, and contraction that leads to postoperative subconjunctival scar. The effect of subconjunctival BVZ and MMC-augmented trabeculectomy in cases of primary open angle glaucoma was beneficial in improving the success rate with better intraocular pressure control and prolonging the trabeculectomy survival with no significant difference between the two groups.

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Introduction

Trabeculectomy is the standard treatment for patients with glaucoma who had failed maximal tolerated medical therapy. Failure of the filtering bleb occurs at different stages after a trabeculectomy. Scarring of the filtering bleb is caused mainly by the proliferation of subconjunctival fibroblasts, the biosynthesis of collagen, and other extracellular materials [1].

The vasculogenesis is a process necessary for supplying oxygen, which is an important nutrient for the scar, it also helps in the migration and proliferation of tenon's fibroblasts that synthesize collagen material, finally leading to a scar tissue [2]. The use of mitomycin C (MMC) reduces fibrosis which in turn increases the possibility of success in filtrating surgery. However, MMC can lead to adverse effects, such as corneal toxicity, hypotony, formation of avascular cystic blebs, leaks, blebitis, and endophthalmitis [3,4].

MMC is an alkylating agent derived from *Streptomyces caespitous* with antineoplastic and antifibroblastic properties, the antifibroblastic activity of MMC has proven to be beneficial to modulate the wound healing after the pterygium excision, refractive surgery, and to reduce cicatrization after trabeculectomy. The concentrations of MMC used during trabeculectomy range from 0.1 to 0.5 mg/ml for 2 min [5–7].

The vascular endothelial growth factor (VEGF) has an important role in scarring as it stimulates angiogenesis and increases vascular permeability, thereby increasing fibroblast proliferation and activity [8]. The bevacizumab (BAV) 1.25 mg/0.1, is a humanized recombinant monoclonal antibody of the immunoglobulin G that joins VEGF and blocks its action in neovascular glaucoma after trabeculectomy. Recent studies have reported an increase in VEGF levels in the aqueous humor [9]. The BVZ has the ability for reducing the fibroblast and its activity *in vitro* [10].

Patients and methods

In this study, 20 patients were divided into two groups: group A included 10 (20 eyes) patients who underwent subscleral trabeculectomy with subconjunctival injection of 1.25 mg/0.1 ml BVZ (Avastin; Genentech Inc., San Francisco, California, USA), and group B included 10 (20 eyes) patients who underwent subscleral trabeculectomy with adjuvant intraoperative use of 0.2 mg/ml MMC for 2 min. This prospective nonrandomized clinical comparative study was conducted in Nour-El-Hayaha Eye Center (Cairo). Patients were diagnosed for primary open angle glaucoma (POAG) with intraocular pressure (IOP) by maximum tolerable antiglaucoma therapy. The study excluded patients with neovascular glaucoma, congenital and juvenile glaucoma, history of ocular pathology, or surgery as retinal surgeries and uveitis. Patients who failed to complete the follow-up examinations after the surgery were also excluded. Written informed consent was obtained after a detailed explanation of the procedure, its possible benefits and risks. Exclusion also included patients with no perception of light or who had previous cyclo-destructive procedures or glaucoma drainage devices.

The demographic data, function of bleb, and ocular examinations results, including measurements of best corrected visual acuity (BCVA) at a distance using a logarithm of the minimum angle of resolution (log-MAR) scale, refractive status using an autorefractometer (KR-8100; Topcon Corporation, Tokyo, Japan), IOP measured by Goldman applanation tonometry (CT-80; Topcon Corporation), and the fundus evaluation using an indirect ophthalmoscope after full pupillary dilatation with tropicamide 1% and phenylephrine 2.5% eye drops were obtained.

Surgical procedure

Sterilization was done by using betadine 10% for the surgical field. Betadine 5% eyedrops were used for the conjunctival cul-de-sac. Application of sterile drops was done. An 8–0 nylon corneal traction suture was used. The conjunctiva was dissected at the supero-nasal quadrant. A 15° knife was used to delineate and a crescent knife was used to dissect and create a half-thickness, 3.5×4.5 mm, rectangular-shaped scleral flap.

Cellulose sponges soaked with MMC (0.2 mg/ml) were applied under the scleral flap, for 2 min and then the surgical area was dried and rinsed with 30 ml balance salt saline (BSS) in group B. Two diagonal scleral flap sutures were preplaced, using 10–0 nylon.

A corneal paracentesis was made by a super blade knife. Sclerectomy was performed with a Kelly-Descemet's punch and the peripheral iridectomy was performed with a Vannas scissors. The scleral flap was approximated with three interrupted 10–0 nylon sutures. The conjunctiva was closed with interrupted 10–0 nylon sutures. Assessment of filtration was done by injecting lactated ringer solution into the anterior chamber (AC) through the paracentesis.

BVZ was injected subconjunctivally adjacent to the temporal edge of the bleb over the scleral flap area, with a 30-G needle. The needle entrance was at least 8 mm away from the bleb to prevent any needle track leakage.

Postoperative care

It included topical antibiotic, topical 1% prednisolone acetate and cycloplegic eye drops for 3 weeks. Ocular massage, laser suture lysis, antiglaucoma medications, and postoperative procedures, such as needling were permitted if necessary, depending on the target IOP.

Postoperative follow-up

There were five postoperative follow-up visits within 6 months: first day, first week, first, 3 and 6 months postoperatively. The primary outcome measures were IOP, BCVA, bleb appearance, number of antiglaucoma medications, postoperative interventions, and complications. The demographic data, function of bleb, ocular examinations results, including measurements of BCVA at a distance using a logarithm of the minimum angle of resolution (log-MAR) scale, refractive status using an autorefractometer (KR-8100; Topcon Corporation), IOP measurement by Goldman Applanation Tonometry (CT-80; Topcon Corporation), and fundus evaluation using an indirect ophthalmoscope were obtained.

Statistical analysis

All statistical calculations were done using (Statistical Package for the Social Science version 20.00; SPSS Inc., Chicago, Illinois, USA). Quantitative data with parametric distribution were done using Analysis of variance *t*-test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The *P*-value was considered nonsignificant at the level more than 0.05, significant at the level less than 0.05, 0.01 and highly significant at the level less than 0.001. Pearson linear correlation coefficient (*r*) was estimated to show the relationship between quantitative parameters.

Results

This study included 20 patients, nine (45%) patients were males and 11 (55%) patients were female. Ages

ranged from 35 to 65 years (mean age 55.2±8.3). The patients were divided into two groups:

Group A: included 10 patients who underwent subscleral trabeculectomy with subconjunctival injection of 1.25 mg/0.1 ml BVZ (Table 1).

Group B: included 10 patients who underwent subscleral trabeculectomy with adjuvant intraoperative use of 0.2 mg/ml MMC for 2 min (Table 1).

The correlation of preoperative and postoperative IOP was recorded on the first day, first, and sixth month, respectively (Table 2).

Table 1 Patients' demographic data

	n (%)	*P*
Male	9 (45)	NS
Female	11 (55)	
Age groups		
35–45	2 (10)	NS
45–55	7 (35)	
55–65	8 (40)	
65+	3 (15)	
Mean±SD	55.2±8.3	
Laterality		
Right eye	25 (62.5)	0.35, NS
Left eye	15 (37.5)	
Duration of symptoms (days)		
Mean	120.3	<0.234
Range	28–365	
Follow-up		
Mean	160.2	0.66[a]
Range	35–290	

[a]Mann–Whitney *U*-test. [b]χ^2-Test.

Preoperative and postoperative visual acuity was recorded and the average percentage change in visual acuity at the first and sixth month postoperation was calculated and the negative sign denoted percentage diminution in the visual acuity. Visual acuity improved during the follow-up period from 10.1 (60 to <−13.5%) in the first month to 32.0 (100–0.0%) in the sixth month in group A, whereas from 9.8 (60 to <−13.5%) in the first month to 34.1 (100–0.0%) in the sixth month in group B, the negative sign denotes percentage diminution in visual acuity (Table 3).

Also the mean±SD value of the IOP in both groups during the follow-up period was recorded. According to the morphological classification, BVZ and MMC groups were also recorded. The incidence of postoperative complications, such as rubeosis iridis and subconjunctival haemorrhage were shown in this study.

The incidence of postoperative complications as cataract formation was statistically significant in both groups but no statistical significant difference between two groups in cataract formation was observed and we noticed no statistical difference between both groups with respect to subconjunctival haemorrhage. Two out of 20 eyes in group A (10.0%) developed failed blebs, whereas group B showed three eyes with failed bleb (15.0%).

Mild hyphema, in one (8.33%) eye in group A, wound leak in one (8.33%) eye in each group, and shallow AC in two (16.7%) patients in each group and was the most common encountered complication in our study.

Table 2 Intraocular tension before and after surgery

IOP	*n* (%)
Tn+	16 (40)
Tn++	14 (35)
Tn+++	10 (25)
Mean±SD	19.8±4.1(10–35)

	P value	
	Group A	Group B
IOP at first day	<0.121, NS	<0.00.1, HS
IOP at first month	<0.18, S	<0.122, NS
IOP at sixth month	<0.98, NS	<0.345, NS
Change at 1 month	<0.001, HS	<0.001, HS
Change at 6 month	<0.001, HS	<0.001, HS

	Postoperative					*P* value
Mean IOP	First day	First to second week	First month	Third month	Sixth month	
Group A	6.6±314	9.9±612	11.2±835	14.5±968	17±845	S
Group B	7.5±433	8.9±612	10.9±785	13.9±881	13.6±623	S

HS, highly significant; IOP, intraocular pressure; S, significant; Tn+, IOP between 23 and 26 mmHg; Tn++, tension between 25 and 30 mmHg; Tn+++, tension more than 30 mmHg.

Table 3 Shows visual acuity preoperative and postoperative at first and sixth month with average percentage change in visual acuity at the first and sixth month postoperatively

Preoperative visual acuity		Postoperative at first month (A) [*n* (%)]			Postoperative at sixth month (B) [*n* (%)]		
		Constant	Improved	Diminished	Constant	Improved	Diminished
6/60	5	1 (20)	3 (60)	1 (20)	–	4 (80)	1 (20)
6/36	8	3 (37.5)	5 (62.5)	–	2 (25)	6 (75)	–
6/24	9	7 (77.7)	2 (22.3)	–	6 (66.6)	3 (33.4)	–
6/18	8	8 (90)	–	–	9 (90)	1 (10)	–
6/12	10	9 (90)	1 (10)	–	9 (90)	1 (10)	–

Median (IQ range)	Group A	Group B
First month	10.1 (60 to <–13.5%)	9.8 (60 to <–13.5%)
Sixth month	32.0 (100–0.0%)	34.1 (100–0.0%)

One (8.33%) patient in group A showed shallow AC with choroidal effusion. One (8.33%) patient in group B developed late bleb-related endophthalmitis after 3 months, which ended in phthisis bulbi (Figs 1–3).

Discussion

VEGF played a key role in the proliferative phase of wound healing, and its levels also increased in the aqueous humour in POAG patients post-trabeculectomy. After application of anti-VEGF antibody, the concentration of VEGF was significantly reduced. Thus, no comparative study had investigated the synergistic effects of intraoperative subconjunctival BVZ injection, in conjunction with MMC, in trabeculectomy [11,12]. A recent study has reported that; increased significant level of VEGF in aqueous humour after trabeculectomy for 30 days postoperatively in animal models [13]. VEGF189 had higher participation in fibrosis; tenon fibroblasts express two VEGF receptors in their membranes, whereas VEGF165 and VEGF121 predominantly regulate angiogenesis [14].

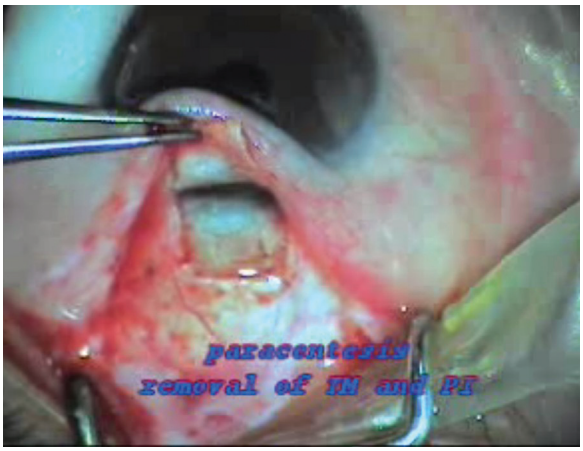
BVZ is a nonselective inhibitor of VEGF121, VEGF165, VEGF189 and therefore it could be efficient for controlling these processes [15].

So, in this study, BVZ was used in comparison to MMC to evaluate postoperative results. After data analysis, a nonstatistical difference between both groups with respect to the mean postoperative IOP, as well as the mean percent reduction in IOP was found. The mean IOP in group A showed an initial drop followed by a gradual rise, whereas in group B the mean IOP showed an initial decline followed by a more or less stable IOP changes. This feature of the course of IOP can be explained by the effect of the MMC, which had prolonged effect than BVZ in suppressing human fibroblast, as observed by some authors [16].

Figure 1

Bilateral diffuse polycystic functional belb.

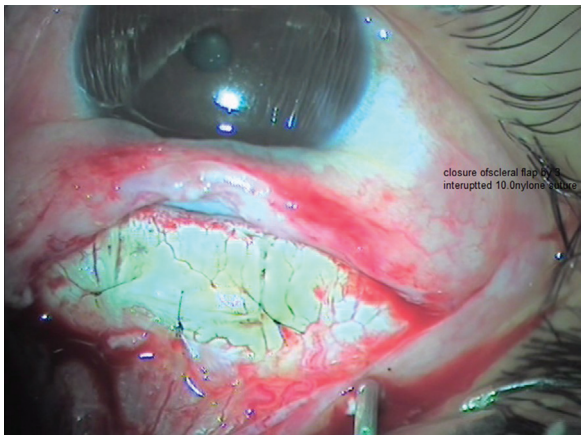
Figure 2



Removal of trabecular meshwork after anterior chamber paracentesis.

In the current study, BVZ is effective and safer adjuvant therapy in subscleral trabeculectomy and though that; its efficacy could be somewhat below that of MMC because a large number of patients in the BVZ group required antiglaucoma medication to reach the target IOP. Although, other studies

Figure 3



Closure of scleral flap by 10–0 nylon suture.

reported this aspect; similar results were observed in trabeculectomy with MMC and that of subconjunctival BVZ [17,18].

Two out of 20 eyes in group A (10.0%) developed failed blebs, whereas group B showed three eyes with failed bleb (15.0%). No significant difference with respect to morphology and vascularization of blebs between both groups were found. Some authors suggested that subconjunctival BVZ injection was effective in adjunctive therapy for reducing the incidence of bleb dysfunction after filtering surgery. They used BVZ 1.25 mg through subconjunctival injection after trabeculectomy and there was a significant reduction in IOP during the first day which increased slightly in the following 3 months. However, their study was limited by the small sample size as well as by the lack of a control group, making it nearly impossible to separate the effect of BVZ from the effect of surgery [19]. After 6 months of follow-up, frequency of 56% of avascular blebs was identified, similar to the studies done by other authors [20].

Trend of bleb vascularity score in our study was similar to the study done by other authors [21]. They studied the use of subconjunctival BVZ (1.25 mg) alone, compared with soaked-sponge BVZ, and with MMC alone, in phacotrabeculectomy. Bleb vascularity of the subconjunctival BVZ group was significantly lower when compared with other groups, 1 month. Clinicians should monitor potential side effects of anti-VEGF agents as their safety profile in glaucoma patients may not be the same as in age related macular degeneration (AMD) patients [22].

In this study, we also noticed that the incidence of postoperative complications as cataract formation is statistically significant in both groups but there is no statistical significant difference between two groups in cataract formation and we noticed no statistical difference between both groups with respect to subconjunctival haemorrhage. Other postoperative complication as rubeosis iridis and ocular hypotony did not occur postoperatively in both groups. This study corresponding to others reported no adverse effects, such as conjunctival necrosis, scleral necrosis, bleb effusion, and hypotony when observed with subconjunctival BVZ injection [23].

The postoperative complications in a study done by other authors found that early hypotony with IOP less than 5 mmHg (in 50% of the patients), cataract development (in 16.7% of the patients), and micro leakage of the conjunctival wound (in 16.7% of the patients). A bleb revision procedure was performed 1 month after trabeculectomy in 16.7% of the patients; neither vessel formation nor adhesion around the scleral flap was observed [24].

Other complications in this study were similar to the study reported by Mahdy *et al.* [24], they reported complications as mild hyphema, in one (8.33%) eye in group A, wound leak in one (8.33%) eye in each group, and shallow AC, in two (16.7%) patients in each group, which was the most common encountered complication in our study. In group A one (8.33%) case of shallow AC led to choroidal effusion. After 3 months, one (8.33%) case in group B developed late bleb-related endophthalmitis, which ended in phthisis bulbi. The complication rate was comparable between the two groups.

Conclusion

No significant difference was found between using subconjunctival BVZ and MMC in management of POAG. The effect of subconjunctival BVZ and MMC was beneficial in improving the success rate with better IOP control and prolonging the trabeculectomy survival by reducing the long-term need of using antiglaucoma medications postoperatively without adding complications.

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Conflicts of interest
There are no conflicts of interest.

Reference

1 Saeed AM, AboulNas TT. Subconjunctival bevacizumab to augment trabeculectomy with MMC in the management of failed glaucoma surgery. *Clin Ophthalmol* 2014; **2014**:1745–1755.

2 Khaw PT, Occleston NL, Schultz G, Grierson I, Sherwood MB, Larkin G. Activation and suppression of fibroblast function. *Eye* 1994; **8**:188–195.

3 Fontana H, Nouri-Mahdavi K, Lumba J, Ralli M, Caprioli J. Trabeculectomy with mitomycin C: outcomes and risk factors for failure in phakic open-angle glaucoma. *Ophthalmology* 2006; **113**:930–936.

4 Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. *Surv Ophthalmol* 2003; **48**:314–346.

5 Lai YH, Wang HZ, Lin CP, Chang SJ. Mitomycin C alters corneal stromal wound healing and corneal haze in rabbits after argon-fluoride excimer laser photorefractive keratectomy. *J Ocular Pharmacol Ther* 2004; **20**:129–138.

6 Hau S, Barton K. Corneal complications of glaucoma surgery. *Curr Opin Ophthalmol* 2009; **20**:131–136.

7 Coppens G, Maudgal P. Corneal complications of intraoperative MMC in glaucoma surgery. *Bull Soc Ophthalmol* 2010; **314**:19–23.

8 Charnock-Jones DS. Vascular endothelial growth factors their receptors and their inhibition. *Cell Transm Newslett Cell Signal Neurosci Res* 2005; **21**:1–5.

9 Tripathi RC, Li J, Tripathi BJ, Chalam KV, Adamis AP. Increased level of vascular endothelial growth factor in aqueous humor of patients with neovascular glaucoma. *Ophthalmology* 1998; **105**:232–237.

10 Li Z, van Bergen T, van de Veire S, van de Vel I, Moreau H, Dewerchin M, *et al.* Inhibition of vascular endothelial growth factor reduces scar formation after glaucoma filtration surgery. *Invest Ophthalmol Vis Sci* 2009; **50**:5217–5225.

11 Yamamoto T, Sakuma T, Kitazawa Y. An ultrasound biomicroscopic study of filtering blebs after mitomycin C trabeculectomy. *Ophthalmology* 1995; **102**:1770–1776.

12 Seibold LK, Sherwood MB, Kahook MY. Wound modulation after filtration surgery. *Surv Ophthalmol* 2012; **57**:530–550.

13 Van Bergen T, Vandewalle E, van de Veire S, Dewerchin M, Stassen JM, Moons L, *et al.* The role of different VEGF isoforms in scar formation after glaucoma filtration surgery. *Exp Eye Res* 2011; **93**:689–699.

14 Memarzadeh F, Varma R, Lin LT, Parikh JG, Dustin L, Alcaraz A, *et al.* Postoperative use of bevacizumab as an antifibrotic agent in glaucoma filtration surgery in the rabbit. *Invest Ophthalmol Vis Sci* 2009; **50**:3233–3237.

15 Chen PP, Yamamoto T, Sawada A, Parrish RK, Kitazawa Y. Use of antifibrosis agents and glaucoma drainage devices in the American and Japanese glaucoma societies. *J Glaucoma* 1997; **6**:192–196.

16 16.Kahook MY. Bleb morphology and vascularity after trabeculectomy with intravitreal ranibizumab: a pilot study. *Am J Ophthalmol* 2010; **150**:399–403.

17 Nilforushan N, Yadgari M, Kish SK, Nassiri N. Subconjunctival bevacizumab versus mitomycin C adjunctive to trabeculectomy. *Am J Ophthalmol* 2012; **153**:352–357.

18 18.Grewal DS, Jain R, Kumar H, Grewal SP. Evaluation of subconjunctival bevacizumab as an adjunct to trabeculectomy a pilot study. *Ophthalmology* 2008; **115**:2141–2145.

19 Sedghipour MR, Mostafaei A, Taghavi Y. Low-dose subconjunctival bevacizumab to augment trabeculectomy for glaucoma. *Clin Ophthalmol* 2011; **5**:797–800.

20 20.Anand N, Arora S, Clowes M. Mitomycin C augmented glaucoma surgery: evolution of filtering bleb avascularity, transconjunctival oozing, and leaks. *Br J Ophthalmol* 2006; **90**:175–180.

21 Sengupta S, Venkatesh R, Ravindran RD. Safety and efficacy of using off-label bevacizumab versus mitomycin C to prevent bleb failure in a single-site phacotrabeculectomy by a randomized controlled clinical trial. *J Glaucoma* 2012; **21**:450–459.

22 Choi JY, Choi J, Kim YD. Subconjunctival bevacizumab as an adjunct to trabeculectomy in eyes with refractory glaucoma: a case series. *Korean J Ophthalmol* 2010; **24**:47–52.

23 Paul H, Jing W. Subconjunctival bevacizumab injection in glaucoma filtering surgery: a case control series. *ISRN Ophthalmol* 2013; **2013**:384134.

24 Mahdy RA, Al-Mosallamy SM, Al-Aswad MA, Bor'i A, El-Haig WM. Evaluation the adjunctive use of combined bevacizumab and MMC to trabeculectomy in management of recurrent pediatric glaucoma. *Eye (Lond)* 2016; **30**:53–58.