

Original research

# Evaluation of topical bevacizumab as an adjunct to mitomycin C augmented trabeculectomy

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## Abstract

**Purpose:** To investigate the safety and synergistic effect of topical bevacizumab after trabeculectomy surgery with mitomycin C (MMC).

**Methods:** In this prospective, non-randomized, comparative interventional study, 40 eyes from 40 patients with uncontrolled open-angle glaucoma were studied after they underwent primary trabeculectomy with mitomycin C (0.02% for 2 min). Following the procedure topical bevacizumab (4 mg/mL) was used for 2 weeks 4 times daily in group A. Patients in group B received routine postoperative care. The outcome measures were the intraocular pressure (IOP), number of anti-glaucoma medications, complications, and bleb evaluation.

**Results:** Of the 32 eyes that had at least 6 months follow-up, 16 were treated with adjuvant topical bevacizumab. The mean preoperative IOP in group A improved from  $26.7 \pm 9.3$  mmHg with  $2.8 \pm 1.3$  anti-glaucoma medications to  $10.5 \pm 2.8$  mmHg with  $0.7 \pm 1$  anti-glaucoma medications at last follow-up ( $P < 0.001$ ). The mean preoperative IOP in group B improved from  $21.8 \pm 6.6$  mmHg with  $3 \pm 0.8$  anti-glaucoma medications to  $11.4 \pm 3.6$  mmHg with  $0.8 \pm 1.2$  anti-glaucoma medications at last follow-up ( $P < 0.001$ ). There was an overall reduction of 54.4% and 43.7% in the IOP in groups A and B, respectively ( $P = 0.18$ ). The cystic type of bleb was less common in group A ( $P = 0.043$ ). One patient in group A developed a streptococcal corneal ulcer 1.5 months after surgery.

**Conclusion:** Administration of topical bevacizumab 4 mg/ml for two weeks following trabeculectomy with mitomycin-C did not significantly affect the IOP trend, but significantly decreased the cystic bleb formation in short-term follow-up.

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**Keywords:** Bevacizumab; Intraocular pressure; Trabeculectomy

## Introduction

Trabeculectomy remains the most common surgical therapy to reduce intraocular pressure (IOP) in patients with uncontrolled glaucoma.<sup>1</sup> The failure of glaucoma filtration surgery

due to excessive scarring remains a major barrier to the control of IOP and arrest of disease progression. Although wound modulation is a life-long process, early surgical results are associated with long-term surgical outcome. After the acute inflammatory phase is over, re-epithelialization, angiogenesis, blood vessel endothelial migration, and granulation occur during the proliferative phase (days 5–14), which are critical factors responsible for bleb failure.<sup>2</sup> There remains a need for safe and effective adjunctive anti-scarring therapy that improves the outcome of filtration surgery, while avoiding vision-threatening complications associated with current anti-scarring agents such as 5-fluorouracil (5-FU) and mitomycin C (MMC).

No conflict of interest was declared.

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Vascular endothelial growth factor (VEGF) is upregulated in the aqueous humor of glaucoma patients and in the rabbit model.<sup>3</sup> Non-selective VEGF inhibition may be more effective in reducing ocular scar formation than selective inhibition of VEGF<sub>165</sub>.<sup>4</sup> Bevacizumab (Avastin; Genentech, San Francisco, CA) is a humanized, non-selective monoclonal antibody against VEGF. The use of bevacizumab in glaucoma is currently an off-label application.

The recent use of intravitreal, intracameral, and subconjunctival anti-VEGF agents for neovascular glaucoma has shown great promise.<sup>5</sup> Repeated subconjunctival injections of bevacizumab were able to reduce both vascularity and fibrosis in a rabbit model for trabeculectomy.<sup>6</sup> Several reports involved the use of intravitreal, intracameral, soaked sponge, subconjunctival (using different doses from 0.2 mg to 2.5 mg) anti-VEGF therapy alone or in combination with other anti-fibrotic agents in trabeculectomy.<sup>1,7–13</sup> Bevacizumab seems to have a worse result than MMC when applied alone in glaucoma surgery.<sup>1,7</sup> Parameters such as dose and route of application may be altered in the future to improve the effect of bevacizumab-augmented trabeculectomy.

Subconjunctival hemorrhage, blebitis<sup>14</sup> and conjunctival necrosis<sup>12</sup> have been reported after subconjunctival injection of bevacizumab. Topical bevacizumab seems to be a relatively safe and effective option to treat corneal neovascularization and is more convenient.<sup>15</sup> Short-term use of topical bevacizumab may be effective in preventing recurrence in a patient with impending recurrent pterygium.<sup>16</sup> Topical bevacizumab was reported to be efficacious in a case of early bleb failure after trabeculectomy.<sup>17</sup> Topically administered bevacizumab, alone or as an adjunct to MMC, after trabeculectomy in rabbit eyes showed a trend towards prolonged bleb survival, even though the results of this study were not statistically significant.<sup>18</sup> Recently, topical bevacizumab (0.25 mg) that was administered 5 times with one-week interval was shown to be useful for the success of trabeculectomy at 6-month follow-up.<sup>19</sup>

This study investigates the safety and synergistic effect of topical bevacizumab after trabeculectomy surgery with MMC compared to standard trabeculectomy with MMC.

## Methods

This prospective, non-randomized, comparative, interventional study was carried out in the glaucoma department of the Farabi Eye Hospital, Tehran University of Medical Sciences in Tehran, Iran, between June 2012 and August 2013. Local ethics committee approval was obtained for the study protocol, and all the patients gave written informed consent.

The inclusion criteria were uncontrollable primary open-angle or pseudoexfoliative glaucoma, where the target IOP had not been reached despite maximum medical treatment. Each patient's ability to comply with study assessments for the full duration of the study was also considered. The exclusion criteria were age <40 years, pregnancy or lactation, previous ocular surgery or trauma, neovascular glaucoma, traumatic glaucoma, aphakic glaucoma, angle-closure glaucoma,

systemic thromboembolic disease, uncontrolled hypertension, diabetic mellitus or congestive heart failure, severe dry eye, or history of corneal ulcer. Any intraoperative complications, wound leakage or shallow anterior chamber noted on the first postoperative day visit were also considered as exclusion criteria. Enrolled eyes were categorized into the two groups. All eyes underwent trabeculectomy surgery with intraoperative application of MMC. The first group comprised 20 eyes treated with topical bevacizumab 4 mg/ml for two weeks in addition of routine postoperative care (Group A), and the second group comprised 20 eyes that received routine postoperative care (Group B). No masking was done, except for the analysis of the bleb morphology.

Preoperatively, full baseline data were obtained for each patient and included best corrected visual acuity (BCVA) with Snellen chart, slit-lamp biomicroscopy, IOP measurement with calibrated Haag-Streit Goldmann applanation tonometer, dilated funduscopy with 90-D lens (cup/disc ratio assessment), and gonioscopy with four mirror Sussman goniolens. BCVA were converted into logarithms of the minimum angle of resolution (logMAR) units using the standard conversion table. The number of anti-glaucoma medications was calculated as the sum of all topical antiglaucoma medications. For the fixed combination eye drops, a score of 2 was assigned.

## Surgical procedure

All operations were performed under topical anesthesia by experienced surgeons. A 7/0 silk superior corneal traction suture was inserted. A fornix-based superior conjunctival dissection was then performed. Following the access to sub-tenon space through limbal dissection, 2% lidocaine without epinephrine was injected through a metallic sub-tenon cannula. The tenon capsule was separated from the episclera backwards by blunt dissection to make a pocket posteriorly. Hemostasis was then achieved. By using a 15-degree knife, a square-shaped superficial scleral flap of 3 × 4 mm was prepared, which was 1/3 of the thickness of the sclera. In all eyes MMC 0.02% (0.2 mg/mL) was applied for 2 min under the scleral flap and to the large surface area between the sclera and tenon capsule, using thinned multiple sponges. The sponges were removed, and the surgical area was irrigated carefully with at least 30 mL of balanced salt solution. The anterior chamber was entered with a 15-degree knife at the temporal cornea through the limbus. The eye was entered with a 15-degree knife and a sclerotomy completed with a vannis, followed by a peripheral iridectomy. The scleral flap was sutured with two releasable 10/0 nylon. The tenon capsule and conjunctiva were closed with 10/0 nylon suture. The anterior chamber was reformed with a balanced salt solution.

## Topical bevacizumab preparation and treatment protocol

A solution of bevacizumab 4 mg/ml was prepared by the pharmacy at Farabi Eye Hospital. Commercially available bevacizumab (25 mg/mL) was diluted in intravenous injections

in 0.9% normal saline to a concentration of 4 mg/mL. The study medication was prepared for each patient at the first postoperative day and stored in sterile, light-protected dropper containers in the refrigerator (4 °C). VEGF is important during the proliferative phase of wound healing so patients in Group A used a prepared medication 4 times a day during the two-week postoperative period. A minimal reported effective dose<sup>20</sup> and short course of usage<sup>21</sup> was considered as the way to minimize potential complications associated with long-term bevacizumab therapy. Postoperative treatment in both groups included topical ciprofloxacin (6 times daily for 2 weeks), topical atropine 1% (4 times daily for 2 weeks) and topical betamethasone eye drops (started Q2h and tapered gradually over 6–8 weeks). Anti-glaucoma medications for the non-operated eye were continued, with the exception of the oral carbonic anhydrase inhibitors.

At the first postoperative visit, assessment of anterior chamber depth and presence of hyphema, bleb evaluation, and Seidel testing were assessed. In the absence of wound leakage, shallow anterior chamber, or any intraoperative complication, patients were divided into two groups according to the availability of topical bevacizumab. Visits in the first postoperative month, especially in the first 2 weeks, included the assessment of visual acuity, IOP measurement with calibrated applanation tonometry, bleb evaluation, slit-lamp biomicroscopy, and funduscopy. Releasable sutures were removed if necessary. Topical bevacizumab usage was assessed in Group A patients, and any complication was recorded, with specific attention given to evidence of uveitis (flare and cells), hypotony, bleb leaks, allergic reaction, unexplained poor vision, corneal changes, and retinal changes. During the follow-up, anti-glaucoma medications were administered depending on the target IOP, and massage of globe was suggested if needed. There were 4 postoperative follow-up visits for comparison of IOP in the two groups: on postoperative months 1, 3, 6, and then at final follow-up. A window of  $\pm 10$  days was allowed for the 3-month visit and of  $\pm 15$  days was allowed for the 6-month visit. At each follow-up visit, BCVA, IOP, bleb morphology, number of anti-glaucoma medications, and any complication or secondary intervention were recorded. At last follow-up visit, bleb photographs were recorded. Bleb morphology was evaluated using a classification based on two factors: vascularity (avascular, normal vessels or congested) and extension (diffuse, flat, encapsulated, or cystic). Complete success was defined as an IOP of more than 5 mmHg and equal to, or lower than, target IOP (18, 15, or 12 mmHg) and at least 20% reduction in preoperative pressure, without any antiglaucoma medications. Qualified success was defined as the above criteria with the addition of antiglaucoma medication. Total success was the sum of qualified and complete success rates. Failure of the treatment was defined as IOP of 5 mmHg or less, or more than target IOP on 2 consecutive visits after 3 months, less than 20% reduction in preoperative IOP, major complication such as loss of vision or endophthalmitis, need for secondary surgical intervention, or needing further glaucoma surgery to control the IOP. Needle bleb revision without the injection of antifibrotic agents was not

considered to be failure of surgery. The time to surgical failure was determined as the duration from surgery to the first event that rendered a patient a surgical failure.

### Statistical analysis

The sample size was chosen to assure a power of at least 80% in detecting a difference of at least 1 mmHg between the groups, with an  $\alpha$  error of 0.05. Based on this estimation, 16 eyes were deemed adequate, and considering 20% as an assumed drop out during the follow-up, recruitment of 20 eyes was targeted for each group. Unfortunately, bevacizumab was not available in about three months interval during study, and the original randomization design of the study was impaired. Therefore, the study is being reported as a non-randomized study.

The data analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL). A one-sample Kolmogorov-Smirnov test was performed to determine whether the data had a normal distribution. Independent and paired *t* tests were used to evaluate between-group and within-group differences, respectively. Categorical variables were analyzed using Pearson  $\chi^2$  tests and Fisher's exact tests. For each group, repeated measures were analyzed using repeated measures of the one-way analysis of variance with Bonferroni correction (IOP) and Friedman test (logMAR). Nonparametric data were analyzed by the Mann-Whitney U test. Kaplan-Meier survival analysis was used to estimate success rates at specific postoperative time points. A *P* value of  $\leq 0.05$  was considered statistically significant.

### Results

Forty eyes from 40 patients were recruited into the study, and 20 eyes received topical bevacizumab in the early postoperative period. Four patients in each group were lost to follow-up by 6 months. One of these patients in Group A was excluded from the study because topical bevacizumab was discontinued after 1 week. This resulted in 16 eyes in each group for final analysis. The groups were similar in terms of preoperative characteristics and demographic features (Table 1). All recruited patients were Caucasian.

The mean follow-up time was  $7.3 \pm 1.3$  months and  $8.3 \pm 1.5$  months in Groups A and B, respectively ( $P = 0.07$ ). Repeated measurement analyses for each group revealed that there were no significant changes in best-corrected logMAR vision from baseline to last follow-up visit (within-group  $P = 0.17$  and between-group  $P = 0.2$ ). The preoperative vision of 3 patients in Group A was reported as hand motion and counting fingers at less than 1 m, and these were not changed to logMAR and assessed as missing data; there were no changes in these patients' vision at last follow-up. No significant difference was detected in terms of best-corrected logMAR vision between the groups at the final visit ( $P = 0.3$ ).

The IOP results for both groups are shown in Fig. 1. The decrease in the IOP between the preoperative visit and the last visit was statistically significant for both groups ( $P < 0.0001$  within-groups and  $P = 0.74$  between-groups). The

Table 1  
Demographic and baseline characteristics of study subjects in the group A versus group B.

	Group A	Group B	P value
No. of patients	16	16	
Mean age $\pm$ SD (y)	64.7 $\pm$ 8	63 $\pm$ 6.7	0.56 <sup>a</sup>
Male/female	16/0	15/1	0.31 <sup>b</sup>
Right/left	7/9	11/5	0.15 <sup>b</sup>
Type of glaucoma			0.07 <sup>b</sup>
Primary open angle	12	7	
Pseudoexfoliative	4	9	
Mean BCVA $\pm$ SD (logMAR)	0.5 $\pm$ 0.3	0.3 $\pm$ 0.2	0.07 <sup>c</sup>
Mean cup/disc ratio $\pm$ SD (vertical)	93.4 $\pm$ 10.7	86.9 $\pm$ 13	0.13 <sup>a</sup>
Mean IOP $\pm$ SD (mmHg)	26.7 $\pm$ 9.3	21.8 $\pm$ 6.6	0.1 <sup>a</sup>
Mean number of antiglaucoma medications $\pm$ SD	2.8 $\pm$ 1.3	3.1 $\pm$ 0.8	0.98 <sup>c</sup>

Group A: topical bevacizumab after trabeculectomy with mitomycin-C; Group B: trabeculectomy with mitomycin-C.

BCVA: best-corrected visual acuity; IOP: intraocular pressure; logMAR: logarithm of minimal angle of resolution; SD: standard deviation; y: year.

<sup>a</sup> Based on independent t-test.

<sup>b</sup> Based on chi-square test.

<sup>c</sup> Based on Mann-Whitney.

preoperative IOP decreased significantly from 26.7  $\pm$  9.3 mmHg to 11.6  $\pm$  3.8 mmHg at month 6, and to 10.5  $\pm$  2.8 mmHg at last visit in Group A, and from 21.8  $\pm$  6.6 mmHg to 12.3  $\pm$  6.0 mmHg at month 6 and to 11.4  $\pm$  3.6 mmHg at last visit in Group B. There was a reduction of 54.4% and 43.7% in the IOP in Groups A and B, respectively, at final postoperative visit ( $P = 0.18$ ). IOP reduction in Group A was 15.46  $\pm$  9.8 mmHg, and in Group B it was 10.43  $\pm$  7.9 mmHg ( $P = 0.13$ ). The number of anti-glaucoma medications dropped from 2.81  $\pm$  1.27 medications before surgery to 0.67  $\pm$  1 medications at last follow-up in Group A, and from 3.06  $\pm$  0.8 medications to 0.81  $\pm$  1.2 medications at last follow-up in Group B. There was no significant difference between the groups in the number of medications at 6 months ( $P = 0.77$ ) and last follow-up visit

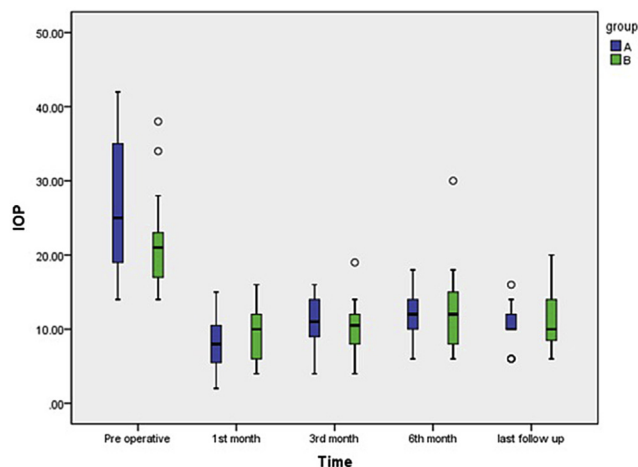


Fig. 1. Graph showing mean and P values of the intraocular pressure (IOP) at each follow-up visit in Group A (topical bevacizumab + mitomycin-C) compared with Group B (mitomycin-C) after trabeculectomy.

( $P = 0.8$ ). On the final visit, 66.7% (10/15) of the eyes in Group A were not receiving anti-glaucoma medication, compared to the 62.5% (10/16) in Group B ( $P = 0.7$ ).

In Group A, from 15 blebs (one missing data) there were 10 blebs (67%) were diffuse; four blebs (27%) were encapsulated, and one bleb (6.7%) was flat. There were no cystic blebs observed in this group. In Group B, from 16 blebs, 7 blebs (43%) were diffuse, 5 blebs (31.2%) were cystic, 3 blebs (18.8%) were encapsulated, and one bleb (6.2%) was flat. No bleb leakage was detected on postoperative follow-up in both groups. Avascular blebs were seen in 6.7% (1/15) and 31.2% (5/16) in Groups A and B, respectively. There were no statistically significant differences in bleb morphology ( $P = 0.1$ ) and vascularity ( $P = 0.24$ ) between the two groups. There was, however, a marginally statistically significant difference between the groups in regard to cystic bleb type when compared with all other types of bleb ( $P = 0.043$ , Fisher's exact tests).

None of the patients experienced systemic complications related to topical bevacizumab. On questioning, none of the patients reported pain, uncomfortable sensations, or unusual or severe postoperative symptoms. The number of postoperative complications was not significantly different in the two groups ( $P = 0.22$ ). While choroidal effusion was detected in 4 patients, there was no hypotony maculopathy in these eyes. The effusion resolved spontaneously in 3 patients in Group B without any need for surgical intervention and choroidal effusion drainage and cataract surgery was done in a patient in Group A, 1.5 months after trabeculectomy. In addition, one patient in Group B underwent surgery due to iris prolapse and shallow anterior chamber. Two cases of increased lens opacity were detected in Group B. One patient in Group A developed a streptococcal corneal ulcer 1.5 months after surgery. Corneal patch graft was undertaken 2 months after surgery (Fig. 2), and he was also considered a failed case, despite this complication possibly being unrelated to postoperative topical bevacizumab. Office-based needle bleb revision without anti-fibrotic usage was used in three patients in Group B, but the needling procedure was not statistically significant different in both groups ( $P = 0.23$ ). Three patients who underwent a second

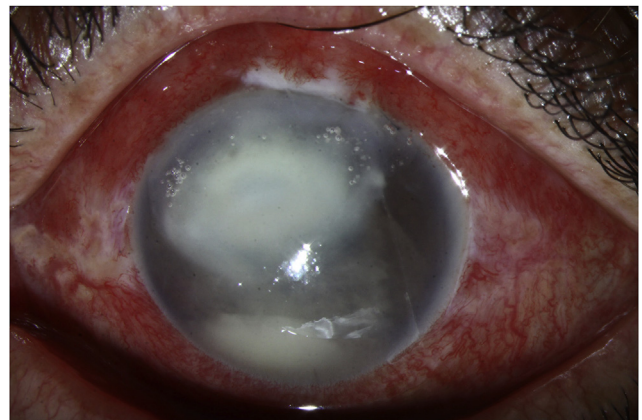


Fig. 2. Corneal ulcer in a case treated with topical bevacizumab after trabeculectomy with mitomycin-C.

intervention due to complications were considered as failed surgery and were not included in final analysis of IOP data.

Although the proportion of eyes considered failures was slightly higher in Group B for each target IOP at last visit, there was no statistically significant difference between the 2 groups for the IOP values of 18, 15, and 12 mmHg (IOP ≤ 18,  $P = 1.00$ ; IOP ≤ 15,  $P = 1$ ; IOP ≤ 12,  $P = 1$ ). Kaplan-Meier survival analysis is shown in Fig. 3 for the target IOP ≤ 12. The IOP values of ≤ 12 mmHg with at least 20% reduction of preoperative IOP, achieved with or without medication, were found in 85.7% (12/14) of Group A and in 73% (11/15) of Group B ( $P = 0.65$ ). Fig. 4 shows the differences in total success between the two groups defined by various IOP criteria at the last visit.

**Discussion**

Wound healing is a cascade of overlapping processes that include hemostasis, inflammation, cell proliferation, and remodeling. In the proliferative phase fibroblasts migrate to the site of injury and re-epithelialization, angiogenesis, and formation of granulation tissue occur.<sup>22</sup> Angiogenesis forms an integral part of wound healing because formation of new blood vessels is necessary for a variety of mediators and regulators to reach the center of the healing process. Important angiogenic factors in ocular neovascularization are VEGF, basic fibroblast growth factor, insulin-like growth factor, and epithelium growth factor.<sup>2</sup> Several treatments and surgical approaches have been developed to successfully modulate scarring after glaucoma filtration surgery.<sup>22</sup> Current anti-scarring agents, such as 5-FU and MMC, reduce the post-surgical scar formation and improve the outcome of glaucoma surgery. However, the use of these agents is associated with severe side effects and complications. Furthermore, blocking transforming growth factor β, which has seemed

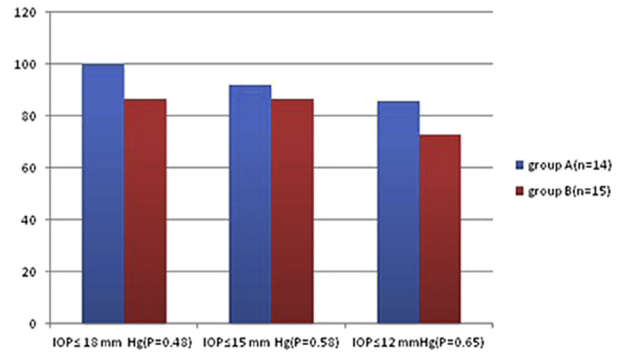


Fig. 4. Proportion of eyes achieving successful outcomes with or without medical therapy (total success) defined by varying intraocular (IOP) criteria. Group A: topical Bevacizumab after trabeculectomy with mitomycin. Group B: trabeculectomy with mitomycin.

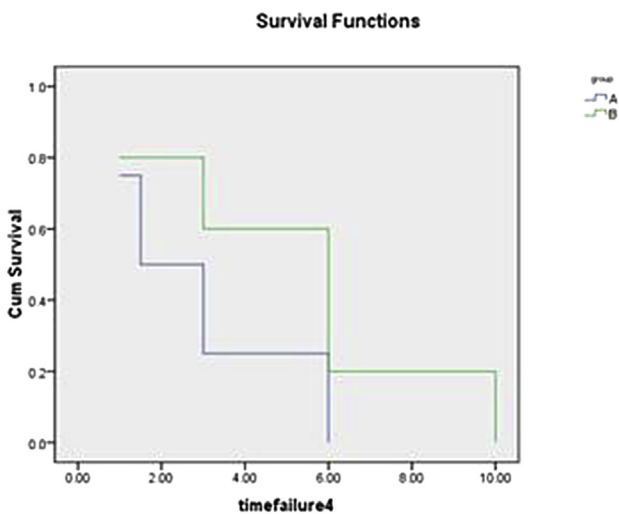


Fig. 3. Kaplan-Meier survival analysis for the target intraocular pressure (IOP) ≤ 12 after trabeculectomy with mitomycin and topical Bevacizumab (Group A) compared with trabeculectomy with mitomycin (Group B).

promising in animal models, has not proved efficient in clinical studies. VEGF<sub>165</sub> and VEGF<sub>121</sub> predominantly affect blood vessel growth, whereas VEGF<sub>189</sub> may be more important in fibrosis. Non-selective VEGF inhibition is anti-angiogenic, as well as antifibrotic, but could not reduce the amount of postoperative inflammatory responses in this trabeculectomy model.<sup>4</sup>

A study by Nomoto et al. investigated the pharmacokinetics of bevacizumab in rabbit eyes using three different routes of administration: intravitreal injection (1.25 mg/0.05 ml), subconjunctival injection (1.25 mg/0.05 ml), and eye drops (1.25mg/0.05 ml six times daily for the first 7 days).<sup>23</sup> Following the administration of eye drops, a small level of bevacizumab was detected in the aqueous humor, iris/ciliary body, vitreous humor, retina/choroid and plasma in the treated and fellow eye but was not effective for the treatment of intraocular neovascular diseases. Systemic exposure to bevacizumab was at the same level when administered by intravitreal or subconjunctival injection and thus suggested that systemic adverse effects of bevacizumab, such as systemic hypertension, thromboembolic diseases might occur after subconjunctival injection, as well as after intravitreal or intravenous injection.<sup>23</sup> Further studies are needed to determine the optimal route for application of bevacizumab for the inhibition of scarring on the bleb area, as well as the optimal time of application and dosage.

In this prospective, non-randomized, comparative study, we assessed the short-term effect of topical bevacizumab on primary trabeculectomy with MMC. At the last visit, we observed an insignificant additive effect on reducing IOP following surgery or antiglaucoma medication usage. Table 2 shows some studies that evaluated the effect of anti-VEGF on primary trabeculectomy. Differences in dosage, time of application, and route of application make comparisons of these studies difficult.

In our study, the cystic bleb was significantly lower in the topical bevacizumab group. Avascular blebs were more common in Group B, but the numbers were not statistically significant. The lack of significance between the postoperative

Table 2  
Some studies that evaluate efficacy and safety of anti-VEGF in primary trabeculectomy; alone or in combination with other antifibrotic agents, different dosages or administration routes.

Study	No.	Route and dosage of anti-VEGF	Conclusion
Nilforushan et al. (2012) <sup>1</sup>	36	Trabeculectomy + MMC versus trabeculectomy + subconjunctival 2.5 mg bevacizumab	Bevacizumab was effective in controlling the IOP however, its effect is less prominent than that of MMC
Grewal et al. (2008) <sup>2</sup>	12	Trabeculectomy with subconjunctival bevacizumab (1.25 mg)	Bevacizumab was potential adjunctive treatment for reducing the incidence of bleb failure
Akkan&Cilsim (2013) <sup>7</sup>	42	Subconjunctival bevacizumab (2.5 mg) versus topical MMC	Bevacizumab was effective and safe in primary trabeculectomy, IOP control appears to be superior with MMC in terms of complete success
Suh&Kee (2013) <sup>8</sup>	36	Intracameral and subconjunctival bevacizumab (1.25 mg) and subconjunctival injections of 5-FU (5.0 mg) versus subconjunctival only 5-FU (5.0 mg).	Bevacizumab may not exert significant additive effects in trabeculectomy when administered in conjunction with 5-FU
Biteli&Prata (2013) <sup>9</sup>	25	1.25 mg bevacizumab subconjunctival after trabeculectomy + MMC	Bevacizumab was safe and effective adjuvant in first-time filtration surgery
Kahook (2010) <sup>10</sup>	10	Group A: trabeculectomy + MMC Group B: trabeculectomy with intravitreal ranibizumab (0.5 mg) and MMC	Combination intravitreal ranibizumab and MMC resulted in more diffuse blebs with less vascularity.
Jurkowska-Dudzińska (2012) <sup>11</sup>	62	5% solution of 5-fluorouracil administered for 4 min versus 1.25 mg of bevacizumab subconjunctivally before and after surgery and again 1 and 7 days after surgery.	No significant differences between the two groups. More patients in the bevacizumab group needed medical therapy.
Sedghipour et al. (2011) <sup>13</sup>	37	Subconjunctival (0.2 mg) bevacizumab versus normal saline	Was not found to affect the trend in intraocular pressure more than placebo

MMC: Mitomycin C, VEGF: Vascular endothelial growth factor.

results may be due to the small sample size of blebs evaluated postoperatively.

An animal study in which 7 postoperative subconjunctival injections of 1.25 mg bevacizumab were compared with subconjunctival 5-FU showed larger and higher blebs with less scar formation.<sup>6</sup> In a pilot study, Kahook observed more diffuse blebs with reduced vascularity in the group with topical MMC and intravitreal ranibizumab compared with topical MMC only.<sup>10</sup> In an interventional case study, Grewal and associates observed that the bleb area decreased over a follow-up period of 6 months, but also that the highest position and vascularity were maintained in cases of injection of 1.25 mg bevacizumab subconjunctivally. The bleb vascularity started to increase 3 months after administration of bevacizumab. This might have prevented the development of cystic avascular blebs.<sup>2</sup> Nilforushan et al. found no statistically significant difference between subconjunctival injections of 2.5 mg bevacizumab versus MMC in primary trabeculectomy.<sup>1</sup> Akkan and Cilsim showed that MMC was more effective in achieving diffuse filtering blebs than subconjunctival bevacizumab as an adjunctive agent in primary trabeculectomy.<sup>7</sup>

Two cases in the topical bevacizumab group failed due to secondary intervention because of complications. The massive choroidal effusion with shallow anterior chamber that was observed in this group after removal of the second releasable suture in the early postoperative period may have been due to delayed healing. Timing of releasable suture removal may need to be delayed after usage of multiple wound healing agents in combination. One case of corneal ulcer occurred in the topical bevacizumab group. This patient was a farmer who smoked and used topical steroid that may have predisposed

him to corneal ulcer. Corneal epithelial healing was delayed by topical application of bevacizumab (2.5 mg/ml) in the experimental model.<sup>24</sup> Galor and Yoo reported a case of melting corneal graft in a patient who underwent penetrating keratoplasty because of idiopathic central corneal perforation. This patient used topical bevacizumab (25 mg/ml) one month before surgery to treat a 360-degree corneal neovascularization and use was continued for 6 weeks after surgery.<sup>25</sup>

Some of the limitations of this study were the small sample size, the short-term follow-up, and non-randomized study design. We did not use any masking except in the final bleb assessment. The surgeries were also not performed by the same surgeon. The mean duration of treatment with anti-glaucoma medication prior to operation was not compared between the two groups. In the assessment of bleb we did not use Moorfields or Indiana bleb grading because slit photography was not standard for such assessments. There are certainly some drawbacks associated with the use of topical bevacizumab, including proper preparation of eye drops, storage, and compliance.

Further prospective, randomized studies are needed to investigate the effect of topical bevacizumab as an adjunct in postoperative care after trabeculectomy with antifibrotic agents to determine its safety, optimal time of application, and dosage. With its stringent criteria for inclusion and success, the study findings may not be applicable to all glaucoma patients; further studies in other glaucoma patients may be needed.

In conclusion, administration of topical bevacizumab 4 mg/ml for two weeks following trabeculectomy with MMC was

not found to significantly affect the IOP trend, but significantly decreased the cystic bleb formation in short-term follow-up.

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