Original Article

Subconjunctival Bevacizumab as an Adjunct to 5-Fluorouracil Enhanced Trabeculectomy: Short Term Results

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Purpose: To compare the results of trabeculectomy with subconjunctival Bevacizumab and 5-Fluorouracil (5-FU); with trabeculectomy with 5-Fluorouracil alone in the short term (i.e. 3 months).

Study Design: Prospective, interventional study

Place and Duration of Study: Department of Ophthalmology, Fauji Foundation Hospital, Rawalpindi, from 18th December 2013 till 16th August 2018.

Material and Methods: A total of 30 eyes (15 in each group) in patients above 40 years of age with primary glaucoma, underwent trabeculectomy with 5-Fluorouracil (5-FU) (50 mg/ml) applied for 5 minutes. At the end of surgery, sub-conjunctival Bevacizumab (Avastin® 2.5 mg in 0.1 ml) was injected in one group. The postoperative IOP, bleb configuration, and complications at 1 day, 1 week, 1 month, and then monthly for 3 months was observed for both groups.

Results: The mean pre-operative IOP in the 5-FU group was 30.6 ± 17.1 mm Hg compared to 28.9 ± 18.9 mm Hg in the 5-FU + Bevacizumab group. The mean IOP of the 5-FU group at 3 months was 13.8 ± 4.25 mm Hg, compared to 12.5 ± 3.37 mm Hg in 5-FU + Bevacizumab group, Comparison of the mean IOP between the two groups revealed lower mean IOP in the 5-FU group at Day 1 ($p = 0.556$) , week 1 ($p = 0.872$), and month 1 ($p = 0.042$), but higher at month 3 ($p = 0.339$). Bleb morphology between the two groups was statistically insignificant ($p = 0.405$).

Conclusion: There is no added benefit of subconjunctival Bevacizumab used as an adjunct to 5-FU enhanced trabeculectomy in the short term.

Key Words: Trabeculectomy, 5-Fluorouracil, Bevacizumab, glaucoma, intraocular pressure.

Success of trabeculectomy for glaucoma may be limited by gradual subconjunctival and episcleral scarring, which causes its failure. The use of antimetabolites like 5-fluorouracil (5-FU) and mitomycin C (MMC) has long been attributed to decrease post-operative scarring[1]. These, too have not been entirely satisfactory. However; the need arises for a newer agent which may enhance the effect of these drugs. Vascular endothelial growth factor (VEGF) is a cytokine, known to be elevated in patients with glaucoma and to promote scarring and angiogenesis during wound healing[1,2]. Bevacizumab (Avastin®) is a full length monoclonal antibody against all isoforms of VEGF-A and has been studied scantily to assess its anti-angiogenic and inhibitory effects on fibroblast proliferation, postoperative scarring and eventual success in glaucoma filtration surgery.[3] Few of those who have studied its effect have presented encouraging results and safety[4,5]. The rationale of this study was to assess the effect of anti-VEGF on the success of trabeculectomy in our local population.

The aim of this study is to compare the effect of subconjunctival bevacizumab as an adjunct to 5-FU enhanced trabeculectomy to the effect of 5-FU alone, mainly in terms of intraocular pressure (IOP) lowering, bleb formation, and complications, in the short term (i.e. three months), and to see if it gives an added benefit.

MATERIAL AND METHODS

A total of 30 eyes in consecutive patients with glaucoma presenting to the operating surgeon, were included in this ongoing study (15 in each group), carried out in the Department of Ophthalmology, Fauji Foundation Hospital, Rawalpindi, which is a tertiary care, teaching hospital affiliated with Foundation University Medical College; from 18th December, 2013 to 16th August, 2018. Approval from the ethical committee was taken. Inclusion criteria were, patients above 40 years of age with primary open angle glaucoma (POAG) or Primary angle closure glaucoma (PACG), and pseudoexfoliative glaucoma (PXF), with uncontrolled IOP after maximally tolerated medical therapy, or non-compliance, or advanced glaucomatous damage at presentation, or as a combined procedure for cataract and glaucoma if on multiple medications. Patient preference for trabeculectomy as a treatment option was also considered if on 2 or more topical anti-glaucoma medications. Exclusion criteria were young patients with glaucoma, congenital, juvenile, secondary, uveitic, traumatic, neovascular, aphakic or patients with ocular surface disease. Pre-operatively, a thorough slit lamp examination was performed of the anterior and posterior segments, along with visual acuity estimation and refraction, Goldmann applanation tonometry, pachymetry and gonioscopy. The patients were assessed and monitored for glaucomatous progression by serial Humphrey perimetry and optical coherence tomography (OCT) of the optic nerve head and retinal nerve fiber layer.

All surgeries were performed under local anesthesia by the author using a standardized technique with facial nerve and retrobulbar blocks. A fornix based approach for trabeculectomy was used with a limbal conjunctival peritomy performed, and then fashioning of a triangular superficial scleral flap measuring 4 x 4 mm with blade # 15, then 5-FU (50 mg/ml) was applied above and below the superficial flap with cotton pledgets for 5 minutes. After thorough washing of the 5-FU, a paracentesis was done, and a deep scleral window was made 1.5 x 2 mm in size with a blade # 11, and a peripheral iridectomy was performed with Vannas scissors. Then the superficial flap was approximated to the sclera with 10/0 nylon sutures one at the apex, and one on the right side with the left side being left unstitched. Then the conjunctiva was sutured with 8-0 silk or 6-0 vicryl on the right side ensuring a water-tight closure. Fluid was injected through the paracentesis to ensure bleb formation and patency of the procedure. In cases of subconjunctival bevacizumab injection (2.5 mg in 0.1 ml), the injection was performed from the left side with a bent needle of a 1 cc (30 G) syringe, horizontally into the bleb 8 mm from the limbus. A subconjunctival antibiotic and steroid injection was given at the end of the surgery. In case of phaco-trabeculectomy, after peritomy and fashioning of superficial flap, phacoemulsification with intraocular lens implantation was performed, after which the trabeculectomy was completed.

The patients were examined by the operating surgeon on the post-operative visits at Day 1, 1 week, 1 month, and then monthly for 3 months. Visual acuity, Goldmann tonometry, and slit lamp examination with fundus assessment was done as routine post operative examination. Bleb assessment using a simple grading system[6-8] was done after healing of the conjunctiva, according to which blebs were classified into four types; Type 1: thin-walled, polycystic (well-functioning), Type 2: Diffuse, flatter and thicker (good functioning), Type 3: Flattened bleb with scarring and little or no function, and Type 4: Encapsulated (Tenon's cyst) with engorged blood vessels and poor function.

The data was analyzed using SPSS version 20. Frequencies and percentages were calculated for all the variables. Unpaired and paired samples t-tests were used for the data analysis as the case may be. The Wilcoxon signed-rank test was used to assess the types of bleb formation between the two groups, as well as complications between the two groups. A p-value of less than *0.05* was considered statistically significant.

RESULTS

A total of 30 eyes of 22 patients were included in this study, with 15 eyes in each group assigned randomly, one group undergoing 'Enhanced trabeculectomy with 5-FU' alone, and the other group undergoing 'Enhanced trabeculectomy with 5-FU + subconjunctival Bevacizumab'. These patients were consecutive patients presenting to the operating

surgeon, who fulfilled the inclusion criteria for trabeculectomy. The majority of patients were females accounting for 21 (95.4%) cases. Primary open angle glaucoma was predominant with 18 (60%) eyes, primary angle closure in 9 (30%) eyes, and pseudoexfoliative glaucoma in the rest of 3 (10%) eyes (Table 1). Enhanced trabeculectomy with 5-FU alone or combined 5-FU with subconjunctival Bevacizumab was performed in 22 (73.3%) eyes and PhacoTrab was performed in 8 (26.7%) eyes.

The mean pre-operative IOP in the 5-FU group was 30.6 ± 17.1 mm Hg (range 15-68), while the mean pre-operative IOP in the 5-FU + Bevacizumab group was 28.9 ± 18.9 mm Hg (range 14-78). However, the difference between the two was not statistically significant (P = 0.758). IOP differences were analyzed at day 1, week 1, month 1, and month 3, and were compared with pre-operative IOP as well as between the two groups (Table 2). The mean IOP of the 5-FU group at day 1 was 10.0 ± 5.8 mm Hg, at Week 1 was 8.9 ± 5.36 mm Hg, at month 1 was 10.6 ± 5.4 mm Hg, and at month 3 was 13.8 ± 4.25 mm Hg. The differences between pre-operative and post-operative IOP in the 5-FU group was significant at all occasions; day 1 (p = 0.001), week 1 (p = 0.001), month 1 (p = 0.001), and month 3 (p = 0.002), thus depicting surgical success in the short term period.

In case of the 5-FU + Bevacizumab group, the mean IOP at day 1 was 11.2 ± 6.8 mm Hg, at week 1 was 9.2 ± 4.25 mm Hg, at month 1 was 16.2 ± 7.39 mm Hg, and at month 3 was 12.5 ± 3.37 mm Hg. . The differences between pre-operative and post-operative IOP in the 5-FU + Bevacizumab group was also significant at all occasions; day 1 (p = 0.005), week 1 (p = 0.003), month 1 (p = 0.042), and month 3 (p = 0.008). This too amounts to successful surgery in the short term.

Comparison of differences in the mean IOP between the two groups revealed lower mean IOP in the 5-FU group at day 1 (p = 0.556), week 1 (p = 0.872), and month 1 (*p=0.042*), but higher at month 3 (p = 0.339). However, only the IOP differences at month 1 were statistically significant between the two groups (Table 3).

Analysis of bleb formation and comparison between the two groups, revealed equal number of cystic bleb formation in the two groups with 5 (33.3%) in each group, with early bleb failure in 2 (13.4%) cases of the 5-FU group, 1 case of a flattened bleb and 1 case of an encapsulated bleb, which required needling (Table 4). However, bleb comparison between the two groups did not reveal statistically significant differences (P = 0.405).

Table 1: Baseline patient characteristics.

Age, years (Mean ± SD)	62.8 ± 7.2
Range, years	50-84
Gender N (%)	22
Male	1 (4.5)
Female	21(95.4)
Eye N (%)	
Right	17 (56.7)
Left	13 (43.3)
Glaucoma Diagnosis N (%)	
POAG	18 (60)
PACG	9 (30)
PXF	3 (10)
Pre-op Anti-glaucoma Medicines	
5-FUø group	
Mean ± SD	2.87 ± 0.35
Range	(2-3)
5-FU + Bevacizumab group	
Mean ± SD	2.93 ± 0.25
Range	(2-3)
Surgical Procedure N (%)	
Enhanced Trabeculectomy	22 (73.3)
PhacoTrab	8 (26.7)

Table 2: IOP§ at different time periods.

IOP§ mm Hg (Mean ±SD)	Pre-op	Day 1	Week 1	Month 1	Month 3
Trabeculectomy with 5-FUø	30.6 ± 17.1 [Maximum 68] [Minimum 15]	10.0 ± 5.8	8.9 ± 5.36	10.6 ± 5.4	13.8± 4.25
Trabeculectomy with 5-FUø + S/C¤ Bevacizumab	28.9±18.9 [Maximum 78] [Minimum 14]	11.2 ±6.8	9.2± 4.25	16.2± 7.39	12.5±3.37

ø 5-fluorouracil § Intraocular pressure ¤ subconjunctival

Table 3: Paired samples t-Test in the 5-FU group & 5-FU + Bevacizumab group.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Pre-Op IOP in 5FU group - Pre-Op IOP in Bevacizumab + 5-FU group	1.66	20.51	5.29	-9.69	13.02	.315	14	.758
Pair 2	IOP Day 1 5FU group - IOP Day 1 Bevacizumab + 5-FU group	-1.13	7.26	1.87	-5.15	2.89	-.604	14	.556
Pair 3	IOP Week 1 5-FU group - IOP Week 1 Bevacizumab + 5-FU group	-.26	6.30	1.62	-3.75	3.22	-.164	14	.872
Pair 4	IOP at 1 month 5-FU group- IOP at 1 month Bevacizumab + 5-FU group	-5.53	9.59	2.47	-10.84	-.21	-2.233	14	**.042**
Pair 5	IOP at 3 months 5-FU group- IOP at 3 months Bevacizumab + 5-FU group	1.33	5.21	1.34	-1.55	4.22	.989	14	.339

Table 4: Bleb Analysis.

Type of Bleb	Trabeculectomy with 5-FU N (%)	Trabeculectomy with 5-FU + S/C Bevacizumab N (%)
Type 1 Cystic	5 (33.3)	5 (33.3)
Type 2 Diffuse	8 (53.3)	10 (66.7)
Type 3 Flattened	1 (6.7)	0
Type 4 Encapsulated	1 (6.7)	0
Z	-.832[b]	
Asymp. Sig. (2-tailed)	.405	

a. Wilcoxon Signed Ranks Test
b. Based on positive ranks.

The number of pre-operative anti-glaucoma medications in both groups ranged from 2-3 (Table 1), and there was a significant reduction of medicines post-operatively in both groups, at 3 months. The comparison for the 5-FU group between the pre-operative medicines (2.87 ± 0.35) and the post-operative medicines (0.20 ± 0.414), was statistically significant (p = 0.000), with only 3 eyes requiring 1 anti-glaucoma agent at 3 months. For the 5-FU + Bevacizumab group, similarly the comparison between the pre-operative anti-glaucoma medicines (2.93 ± 0.25) and post-operative medicines (0.27 ± 0.458), were statistically significant (p = 0.000), with 4 eyes requiring 1 drop at 3 months. The difference in anti-glaucoma agents at 3 months between the two groups was not statistically significant (p = 0.334).

A few early complications were encountered in both groups, summarized in Table 5, with a slightly higher number in the 5-FU + Bevacizumab group, but the differences between the two groups were not found to be statistically significant (p = 0.373). Shallow

Table 5: Complications of Trabeculectomy.

Complications Trabeculectomy with 5-FU	**N (%)**
Hyphema	1 (6.7)
Failure of filtration	2 (13.3)
Bleb leak	2 (13.3)
Trabeculectomy with 5-FU + S/C Bevacizumab	**N (%)**
Epithelial defect	1 (6.7)
Imperforate PI[a]	1 (6.7)
Hyphema	1 (6.7)
Shallow AC[β] *& Choroidal detachment*	1 (6.7)
Peaked pupil	1 (6.7)
Posterior synechiae	2 (13.3)

Z	-.891[b]
Asymp. Sig. (2-tailed)	.373

a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.

α Peripheral iridectomy β anterior chamber

anterior chamber (AC) was considered only if iris cornea touch was present in the mid-peripheral iris and this was present in 1 (6.7%) case which led to choroidal detachment, in the 5-FU + Bevacizumab group, which resolved with steroids and cycloplegics. Also 1 case of imperforate peripheral iridectomy (PI) required Nd: YAG iridotomy post-operatively, also in the same group. Bleb leaks were found in 2 (13.3%) cases of the 5-FU group, managed by bandage contact lenses (BCL). The complications encountered did not have a considerable long lasting effect in terms of success.

DISCUSSION

The results of our study indicate no added benefit of subconjunctival bevacizumab used as an adjunct to 5-FU enhanced trabeculectomy, in terms of IOP lowering, as the mean IOP in the 5-FU group was lower at all occasions except at month 3. Similarly, no significant differences in bleb formation or complication rate were seen on comparison of the two surgical groups. Mean post-operative IOP was significantly lower in both groups at all times, when compared to the pre-operative IOP.

A vascularized bleb is long known to cause trabeculectomy failure. Failure of trabeculectomy is invariably caused by subconjunctival and episcleral fibrosis, which is the result of myofibroblast transformation[9] triggered by vascular endothelial growth factor (VEGF), by the induction of transforming growth factor (TGF)-β1. Thus factors inhibiting VEGF would result reduce this fibrosis. Wound modulation by subconjunctival bevacizumab in rabbits undergoing trabeculectomy was demonstrated in 2014 by Ozgonul[10] and colleagues, who concluded it to be superior to subconjunctival 5-FU. Evidence in literature initially suggested adjunctive treatment of bevacizumab in trabeculectomy to be promising as indicated by Frieberg et al[1] in 2013, who used this agent and it reduced the number of post-operative injections of 5-FU. However, similar to our study, no statistically significant reduction in IOP, bleb morphology or complications was observed. Sedhipour et al[2] in 2011 assessed the short-term benefit of bevacizumab and found no significant difference when compared to placebo, similar to our results. Jukowska-Dudzińska[11] et al found more patients with the bevacizumab group needing medical therapy at 1 year, compared to 5-FU, the rest of results were similar to ours. Nilforushan[12] et al in 2011, found benefit of bevacizumab alone on IOP lowering, but less than that of mitomycin alone. Bitelli[5] et al found it to safe and effective adjuvant therapy with MMC. Suh et al[13], Kiddie[3] et al, Saeed[8] et al and Elgin[14] and coworkers, did not find additional additive effects. The effectiveness and safety of this drug was assessed by Akkan[15] et al in 2015, which appeared to be safe but not superior to MMC. Mild central bleb avascularity was observed with subconjunctival bevacizumab by Chua[16] and coworkers, but not significantly so. A meta-analysis of randomized controlled trials carried out in 2016 by Liu X et al[17] found bevacizumab to be superior compared to placebo, but no difference was seen when used in conjunction with MMC versus MMC alone, and it was found to increase the rate of bleb associated complications like bleb leaks and encysted blebs, compared to MMC. In our study however, on the contrary, bleb leaks and encysted bleb was seen in the 5-FU group. Wang[18]et al used subconjunctival bevacizumab as an adjunct to MMC and found no benefit as well.

Contrary to this, in 2017, adjuvant bevacizumab was found to be comparable to MMC in the long term, in primary open angle glaucoma, along with demonstrating significant bleb avascularity, as noted by Kaushik[19] et al in India. Similarly, Popescu[20] has claimed its positive role in inflammatory glaucoma.

Cheng[21] and coworkers did an extensive electronic database search on randomized controlled trials

comparing subconjunctival bevacizumab to other agents, and concluded in 2016, that there is insufficient and low quality evidence to support or refute its use for wound healing in glaucoma surgery.

Strengths of our study are that this is the first of its kind in Pakistan. Equal allotment of cases and good follow up has been ensured. A standard technique with a single surgeon and patient assessment has also been standardized.

Limitation of our study is small sample size. Our department does not have a separate glaucoma facility and collecting patients has taken a lot of time. These patients will be continued to be followed up for long term results. Future work required is larger scale studies with more study subjects and long term assessment.

CONCLUSION

Subconjunctival bevacizumab in conjunction with 5-fluorouracil enhanced trabeculectomy offers no additional benefit in terms of IOP lowering, bleb morphology or post-operative complications; when compared to 5-FU enhanced trabeculectomy alone, in the short term.

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