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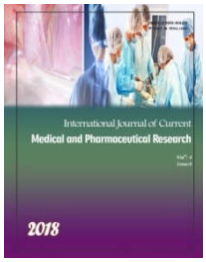

COMPARATIVE EVALUATION OF POST-OPERATIVE CHANGE IN ENDOTHELIAL CELL COUNT USING SUBCONJUNCTIVAL MITOMYCINC OR BEVACIZUMAB AS AN ADJUNCT TO TRABECULECTOMY: A PILOT STUDY

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ABSTRACT

Purpose: Mitomycin-C (MMC) is an useful adjunct in high risk glaucoma surgery. Few clinical data both supporting and negating the role of Mitomycin C exist regarding potential deleterious effect of MMC on corneal endothelium. Subconjunctival or intracameral Bevacizumab is used as an adjunct to trabeculectomy. Bevacizumab has been found to be relatively safe when used intracamerally or intravitreally. No study has reported effect of Subconjunctival Bevacizumab on corneal endothelium in patients undergoing trabeculectomy. We intend to compareand also retrospectively analyze the two groups from past clinical data on Central endothelial cell density (CECD) postoperatively.
Methods: It is prospective, randomized study done on forty eyes of thirty-five adult patients with POAG or PACG were randomized to two groups, A and B respectively. Group-A underwent trabeculectomy with subconjunctival injection of Bevacizumab at the end of surgery adjacent to the bleb. Group B underwent trabeculectomy with subconjunctival 0.2mg/ml MMC applied for 2 minutes. Preoperative and postoperative CECD at day 7 and day 120 were analysed.
Results: Pre-operative, post-operative day 7 and day 120 specular counts were 2318.97±422.23/mm^2, 2177.13±394.24/mm^2 and 2170.3±411.78/mm^2 respectively in Group A and pre-operative, post-operative day 7 and day 120 post-op specular counts were 2420.56±513.79/mm^2, 2175.11±478.48/mm^2 and 2148.94±451.14/mm^2 respectively in Group B. There was significant decrease in the two groups postoperatively however the intergroup variation was not significant
Conclusion: There is a significant loss of corneal endothelial cells three months after trabeculectomy with both Subconjuctival Bevacizumab and Mitomycin C. However, post trabeculectomy endothelial cell loss appears to be multifactorial in nature and not solely due to adjuncts.

INTRODUCTION

Since its introduction by Cairn's in 1968, Glaucoma Filtering Surgery (GFS) has been the mainstay of surgical treatment for glaucoma which is either medically uncontrolled or where the intraocular pressure (IOP) continues to be persistently high despite relief of the pupillary block[1]

Use of adjuncts to modulate bleb wound healing is a common procedure in modern day GFS in order to increase the bleb survival. Pharmacological modulations aim to intervene in the sequential event involved in normal wound healing response. Modulators currently used include antimetabolites like 5-Flurouracil (5-FU) and Mitomycin C (MMC) which cause widespread apoptosis and inhibition of fibroblast function[2,3]Mitomycin C augmented trabeculectomy is the most common GFS practiced worldwide in high risk cases[3]. Derived from Streptomyces Caepitosus, a soil fungus, MMC is a non-specific cell cycle alkylating agent, which inhibits various steps of cell division like DNA replication, mitosis and protein synthesis. It increases the chance of GFS success by directly inhibiting fibroblast and endothelial cell growth and replication, which play an important role during the proliferative phase of wound healing pathway[5]. It exerts its most profound cellular toxicity in the late G1 and early S cellular phases[5].In high doses, MMC has a direct cytotoxic effect which is independent of cell cycle.[6] MMC was first used as an adjunct in GFS by Cheung *et al*[4].

There is a high possibility that MMC can permeate tissues

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beyond its area of application and can have cytotoxic and apoptotic effects even on non mitotic cells like corneal endothelium[8].Wu Ky *et al* have shown direct cytotoxic effect of MMC on cultured endothelium and keratocytes[7,8]. MMC usage during trabeculectomy has been reported to be associated with 10-11% greater endothelial cell loss than no antifibrotic use.[9]

Inhibiting specific pathways of wound healing at molecular and immunological level is associated with less side effects and ocular toxicity. Some promise is being offered by the newer generation of products that are aimed at preventing angiogenesis and fibrosis at immunological level such as inhibitors of Transforming Growth Factor β2 (TGF-β2), Matrix Metalloproteinase Inhibitors (MMPI)[10] and Anti Vascular Endothelial Growth Factor (Anti-VEGF). Although in their initial phase they have shown promising results.

Angiogenesis is an integral part of wound healing process during proliferative phase. It is characterized by new vessel formation and vascular hyper permeability bringing increased oxygenation and nutrient delivery, which promotes cellular and matrix deposition in the wound. Well vascularised blebs have been associated with poorer prognosis than less vascular blebs[12].Bevacizumab has been shown to cause a dose dependent suppression of cell proliferation. Subconjunctival bevacizumab has been successfully used for bleb modulation post trabeculectomy and establishing comparable IOP control similar to MMC modulated trabeculectomy.[11, 13,14]

The aim of this study was to evaluate the effect of MMC on corneal endothelial density in comparison to Subconjunctival Bevacizumab and their correlation.

MATERIALS AND METHODS

This study was a prospective, randomized, interventional clinical trial at the Glaucoma services of Guru Nanak Eye Centre, Delhi. All patients provided informed consent after the aim of the study and possible risks had been fully explained. The study was conducted in compliance with the tenets of Declaration of Helsinki and ethics committee approval was obtained before the start of the study.

Following Inclusion and Exclusion criteria were laid down.

Inclusion Criteria

Patients of Primary Open Angle Glaucoma and Primary Angle Closure Glaucoma fulfilling the following criteria were included

1. IOP>21 mm of Hg with at least two topical anti glaucoma medication.
2. Changes in the optic disc or in Visual Field that is suggestive of progressive glaucomatous damage inspite of IOP<21 mm of Hg.
3. No previous history of intraocular surgery or any surgery on conjunctiva.
4. Patient willing to give consent, for follow-up and investigations.

Exclusion Criteria

1. Any form of secondary glaucomas in the form of uveitic glaucoma, neo vascular glaucoma, lenticular, pigmentary and pseudo exfoliation glaucoma.
2. History of prior surgical interventions in the form of past glaucoma surgery, cataract surgery, or any other surgery involving conjunctiva.
3. Existing conjunctival disease.
4. History of hypertension, coronary artery disease, patient on anti-coagulation therapy (warfarin) and any past cerebro-vascular accident.
5. Pregnant, lactating or pre-menopausal women not using adequate contraception.
6. Any known allergies to Mitomycin C or Bevacizumab.
7. Based on Inclusion and Exclusion criterion 40 eyes of 35 patients were included in the study.

Primary Open Angle Glaucoma and Primary Angle Closure Glaucoma was defined on the basis of history of presenting illness, systemic history, IOP by applanation tonometry, Gonioscopy, evidence of glaucomatous disc damage on direct ophthalmoscopy and slit lamp biomicroscopy by 90 Dioptres lens, slit lamp examination and posterior examination to rule out any secondary cause of glaucoma and visual field examination wherever possible by Humphrey Visual Field Analyser. Inability to control IOP with at least two topical anti glaucoma medications in POAG patients or after relieve of pupillary block in PACG patients was indication for surgery.
The patients were >35 years of age and diagnosed case of Primary Glaucomas. The patients were randomly divided into two groups on satisfying the inclusion and exclusion criterion. Allocation of patients into two equal groups, of 20 eyes each, was done randomly using computer software (Microsoft Excel). Thus they were divided into,

Group A: comprising of 20 eyes, received Bevacizumab as Subconjunctival Injection at the end of trabeculectomy.
Group B: comprising of 20 eyes, received peroperative Mitomycin C (MMC).

Specular Count

Endothelial cell count was done pre-operatively and post-operatively on day 7 and day 120 and count was analyzed. Specular counts were done by Topcon SP-3000P specular microscope using variable frame analysis.

Surgical Technique

Peribulbar anesthesia was given with lignociane and bupivacaine in equal proportions. Globe traction was achieved with limbo corneal sutures using 6-0 silk. Limbus based conjunctival flap was made 7-8mm behind the limbus conjunctiva and Tenon's capsule was gently dissected to reach the limbus. Light cautery on exposed sclera was used to achieve haemostasis. Scleral flap 4×4mm was than dissected till the entry into clear cornea. A thorough wash with 20% Ringer Lactate was given. After resection of anterior trabecular block (2.5×1mm) a peripheral iridectomy was performed. Scleral flap was sutured with 10-0 nylon sutures at two corners. Tenon's capsule was sutured with interrupted 8-0 vicryl sutures. Conjunctiva was sutured with 8-0 continuous silk sutures. Subconjuctival Gentamycin+Dexamethasone(0.5ml+0.5ml) was given in the inferior fornix in all subjects. Post operative topical corticosteroids (Prednisolone acetate) and antibiotic drops were given4 to 6times/day for a maximum period upto 6 weeks.

- Group A subjects received 0.05 ml (1.25mg) of Subconjuctival Bevacizumab adjacent to the bleb area at the end of the surgery with a 30 gauge needle and tuberculin syringe
- Group B subjects received MMC by merocel sponge soaked in 0.2mg/ml of MMC under the conjunctiva and under the tenonfor a period of 2 minutes.
- The postoperative regimen consisted of antibiotic and steroid drops every four hours for 1 week, which was tapered over the first one and half postoperative month. Cycloplegic drops were given every eight hours for the first week.

Postoperative Evaluation

All patients were followed up for a minimum period of 4 months post surgery. Patients were followed up on day 1, 7, 14, 30, 60, 90 and at 120 days with ± 7 days for last four visits post-operatively.

Endothelial cell count was done post-operatively on day 7 and day 120.

Statistical Evaluation

Statistical analysis was performed using Statistical Package for Social Sciences software version 17 (SPSS Inc., Chicago, Illinois, USA).

For all measurements student's T test and a two-tailed test was used, and $p< 0.05$ was considered as significant for measured variables.

RESULTS

This study was conducted on a total of 40 eyes which were further divided by randomization into groups A and B, each group consisting of 20 eyes each.

Age of included subjects ranged from 35-65 years. Mean age in Group A was 50.05±8.33yrs and Group B was 47.95±6.83yrs and the overall mean age of all the patients was 49±7.59yrs. No statistically significant difference was found in age distribution between the two groups, $p=0.388$ ($p>0.05$) using student's t test.

Of the 40 eyes, 17 eyes were female and 23 eyes were of male patients, with a male to female ratio of 4:3 (Fig.2). Both the groups were matched in terms of sex distribution, $p=0.388$ ($p>0.05$).

Out of 40 eyes, 28 eyes were diagnosed cases of PACG and 12 eyes were of POAG. Group A had 6 and 14 eyes of POAG and PACG respectively. Group B had 6 eyes of POAG and 14 eyes of PACG. Both groups matched regarding type of glaucoma selection, $p=1$ ($p>0.05$) using student's t test.

BCVA was determined by Snellen's acuity chart and expressed in Logmar unit by using Snellen to logmar conversion tables, for the purpose of statistical analysis. Pre-operative mean BCVA in Group A and Group B was 0.62±0.55 and 0.57±0.43 logmar units respectively, $p=0.99$ ($p >0.05$) which was statistically not significant.

Mean postoperative BCVA on day 120 was 0.612±0.51 and 0.587±0.39 logmar units in Group A and B respectively with p value=0.763 ($p>0.05$) using student's t test

Mean pre-operative IOP in both groups was found to correlate well with a mean of 35.55±8.8 mm of Hg and 35±5.80 mm of Hg in Group A and Group B respectively, $p=0.817$ ($p>0.05$) using student's t test.

Post-operative IOP was measured on days 1, 7, 14, 30, 60, 90 and 120. In both Groups, post-operatively there was a significant fall in mean IOP post-operatively which was maintained throughout the period of observation post-operatively.

Endothelial Cell Count

Endothelial cell count was measured using specular microscopy.

Pre-operative, post-operative day 7 and day 120 specular counts were 2318.97±422.23/mm^2, 2177.13±394.24/mm^2 and 2170.3±411.78/mm^2 respectively in Group A. Specular count decreased significantly post-operatively as compared to pre-operative , $p=0.000$($p<0.05$) and $p=0.001$($p<0.05$) in both the pairs respectively using student's t test.

In Group B mean pre-operative, post-operative day 7 and day 120 post-op specular counts were 2420.56±513.79/mm^2, 2175.11±478.48/mm^2 and 2148.94±451.14/mm^2 respectively. When assessed for the post-operative change in specular in comparison to pre-operative specular, $p=0.000$($p<0.05$) and p0.000 ($p<0.05$) in both the pairs using student's t test.

In both groups there was significant decrease in specular counts post-operatively which was observed till day 120. There was no significant effect on corneal clarity in any eye.

Distribution of specular count pre-operatively in both groups was comparable, $p=0.499$($p>0.05$). Post-operative day 7 and day 120 specular count were also comparable between the two groups, $p=0.989$($p>0.05$) and $p=0.625$($p>0.05$) using student's t test (Table 1).

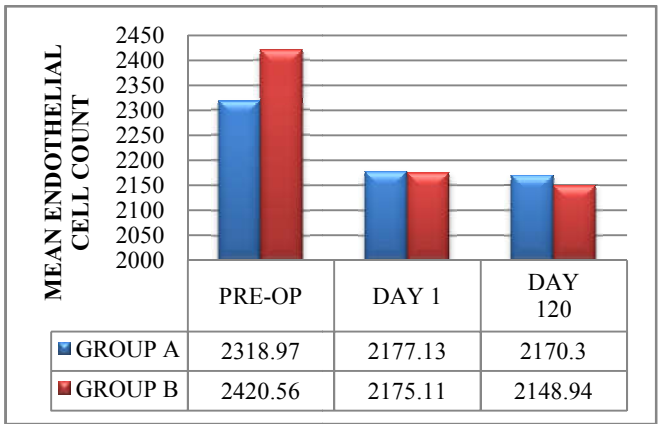

Table 1 Mean specular count (no. of cells/mm^2) in Group A and Group B

DISCUSSION

Corneal endothelial cells are amitotic cell layer, functional and physiological integrity of which is of utmost importance for maintaining corneal transparency. Corneal endothelial cells are lost over time and no new cells replace the lost cells. Post endothelial cell loss, remaining endothelial cells enlarge and slide to cover the defect.[15,16]

There have been concerns in the past that Mitomycin C augmented trabeculectomy which is the current gold standard can permeate the ocular tissue although applied subconjunctivally and can cause global loss of cells especially corneal endothelium and can be Achilles heel for Trabeculectomy augmented with Mitomycin C[9,17] . Pastor and

colleagues in their study published in 1993, raised concerns about Mitomycin C augmented trabeculectomy after single intraoperative application of MMC. Percentage cell loss ranged from 4.7% to 20% postoperatively with an average of 11.4%±5.36 [17]. In a study published by Sihota *et al* comparing three groups of 10 eyes each with trabeculectomy alone, trabeculectomy with MMC (0.2mg/ml) and trabeculectomy with MMC (0.4mg/ml), they found a significant difference between MMC used group and MMC naïve group. The percentage cell loss in Group I was 3.73±2.73%, in Group II 13.90±4.69% and in Group III 14.52±7.8%. They concluded that MMC whether used at concentration of 0.2 mg/ml or 0.4 mg/ml caused significantly higher postoperative endothelial cell loss than compared to trabeculectomy alone[9].

MMC augmented trabeculectomy is one of the preferred GFS in patients with high risk of future Bleb failure. This study was subgroup analysis of a larger study whose primary aim was to compare efficacy and associated complications of sub-conjunctival Bevacizumab versus standard subconjunctival Mitomycin C for bleb modulation in terms of IOP control. During our literature search we came across various studies, which have red flagged Mitomycin C safety on long term corneal Endothelial Health.

We intend to compare the post operative endothelial cell loss in the two groups as a byproduct of this study, to get a bigger picture in terms of various red flags that have been raised in past regarding overall safety of Mitomycin C versus subconjunctival Bevacizumab.

Anti-VEGF molecules are one of the commonly administered drugs in ophthalmic practice. Their main route of administration are intravitreal, intracameral, subconjunctival and topical. In our study group with subconjuntival Bevacizumab augmented trabeculectomy we injected 0.05ml (1.25mg) subconjuctivally adjacent to the bleb with the injection stream aiming towards the conjuctival fornix post-operatively. Although the direction of aqueous flowin the bleb would be ideally from high pressure system i.e(Anterior chamber) to a low pressure system i.e (subtenons). Retrograde flow of Bevacizumab to the Anterior chamber is highly unlikely. However, it's wise to assume that anti-VEGF molecules can diffuse into anterior chamber.

In a study done on rabbit eyes, Park *et al* found acceptable level of safety of intracameral Bevacizumab (ICB) on corneal endothelial cells[19]. Hosny *et al* studied 10 patients of Neovasular glaucoma injected with intracameral Bevacizumab (0.05ml,1.25mg Bevacizumab). In their study mean endothelial cell count was 2631.5 cells/mm ±412.59 (range 1718-3048) which dropped to 2547.6 cells/mm2 ± 503.86 (range 1319-2993) after ICB injection. The mean endothelial cell loss was 3.95% ±6.78 (range 1.54-23.22) which was statistically significant (p=0.02, paired sample t-test), yet it had no clinical effect on the cornea[18].

One of the commonest procedures in ophthalmic practice is Intravitreal Anti-VEGF injection, which is done worldwide. Post intravitreal Anti-VEGF injection, aqueous humour VEGF levels are significantly decreased and injected Anti-VEGF molecules can be easily detected on aqueous analysis. Guzel and colleagues analysed effect of Intravitreal Bevacizumab and Ranibizumab in 30 eyes each of Diabetic Macular Edema. In the Bevacizumab group they successfully concluded that monthly injection of Bevacizumab has no significant deleterious effect on endothelial cell count.Endothelialcell count after three injections increased from 2234.5 to 2267.3 cells/mm^2 in the IVB group and from 2198.9 to 2231.6 cells/mm^2 in the IVR group, although not statistically significant (p = 0.74 in the IVB group and p = 0.66 in the IVR group).[20]

It appears that decrease in endothelial cell count in Bevacizumab treated eyes is more related to intracameral procedure performed rather than presence of Bevacizumab molecule in Aqueous. It can also be hypothesized that at a certain Aqueous concentration above which Bevacizumab can cause deleterious effect on endothelial cell count.

Specular count was the most important parameter in our study to evaluate corneal health.

In Bevacizumab group mean pre-op specular count was 2318.97±422.23/mm^2. Day 7 and day 120 post-op specular count were 2177.13±394.24/mm^2 and 2170.3±411.78/mm^2 respectively. Specular count decreased significantly post-operatively when compared from pre-operative specular to day 7 and day 120, p=0.000($p<0.05$) and p=0.001($p<0.05$).

In MMC Group mean pre-op, day 7 and day 120 post-op specular were 2420.56±513.79/mm^2, 2175.11±478.48/mm^2 and 2148.94±451.14/mm^2 respectively. When assessed for the post operative changes in specular count in comparison to pre-operative specular both the pairs day 7 to pre-op and day120 to pre-op were statistically significant.

In our study there was significant decrease in specular count till 3 months in both Group A and B. Study done by Hosny *et al* on effect of Intracameral Bevacizumab Injection on Corneal Endothelial Cells showed a postoperative drop to 2,547.6± 503.86/mm[18] from a mean preoperative endothelial cell density of 2,631.5 ± 412.57/mm^2, p= 0.04 using paired sample t-test[18]. Sihota *et al* examined 30 eyes which underwent trabeculectomy and showed that at 3 months post-operatively eyes with plain trabeculectomy had 3.73±2.73%, with intraoperative 0.2mg/ml MMC had 13.90±4.69% and eyes with intraoperative 0.4mg/ml MMC had 14.52±7.8% loss of endothelial cells when compared to pre-operative values[9].

Both group here showed a decline in the specular counts, which was dramatic on postoperative day 7. The decline maintained till the end of study. It is worth mentioning that the drop in count did not translate into lack of corneal transparency and, thus supporting the safety of use of either drug in our study. Observation in Group A and B are comparable and reaffirms the work done by Hosny *et al* regarding Bevacizumab and Sihota *et al* with intraoperative use of Mitomycin C.

However, our study clearly demonstrates that amount of endothelial cell loss was comparable between the two groups and there was no statistically significant intragroup variation at day 7 and 120.

Several mechanisms have been implicated to cause decrease in endothelial cell count post trabeculectomy which include high IOP, shallow anterior chamber pre-operatively or post operatively, intraoperative loss, hypoxic damage due to impaired aqueous flow, toxicity of anti-glaucoma drugs, and congenital alteration of corneal endothelium in glaucoma patients and use of Anti-metabolites for bleb modulation[21].

Around 7-10% loss of cells has been seen in trabeculectomy without use of any adjuncts at 3 months follow up[22]. Chronic inflammation due to bleb, migration of corneal endothelial cells into the bleb and subsequent death and aqueous jet stream injury are the various hypothesis that have been implicated but need to be evaluated[23].

In our study, both the groups parallel the endothelial cell count cell loss and there is no significant intragroup variation. The MMC group cell loss is almost similar to various studies done in past which have shown around 11% decrease in central endothelial cell count at 3 months followup. Not much literature is available on health of corneal endothelium with respect to post-operative administration of Subconjunctival Bevacizumab for bleb modulation. It appears though that mechanism of corneal endothelial loss is amulti-factorial process in patients undergoing Glaucoma Filtering Surgery.

CONCLUSION

Mitomycin C augmented trabeculectomy has been associated with thin leaking blebs, blebitis, scleromalacia, punctate epithelial defect and post operativehypotony. Sub conjucnctival Bevacizumab used as an adjunct at the end of surgery can have equal success rates in term of IOP control without associated complications. Although, in our study MMC augmented trabeculectomy results in higher mean cell loss than Bevacizumabpost-operatively the difference between the two groups is not statistically significant which further supports multifactorial nature of post trabeculectomy decrease in CECD.

Refrences

1. Choong YF, Irfan S, Menage MJ. Acute angle closure glaucoma: an evaluation of a protocol for acute treatment. *Eye* 1999;13:613-6.
2. Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. *Ophthalmology* 1991;98:317-21.
3. Kitazawa Y, Kawase K, Matsushita H, Minobe M. Trabeculectomy with mitomycin. A comparative study with fluorouracil. *Arch Ophthalmol* 1991;109:1693-98.
4. Cheung JC, Wright MM, Murali S, Pederson JE.Intermediate-term outcome of variable dose mitomycin C filtering surgery. *Ophthalmology*. 1997;104:143-9
5. Dorr RT. New findings in the pharmacokinetic, metabolic, and drug-resistance aspects of mitomycin C. *SeminOncol*1988;15:32-41.
6. Khaw PT, Sherwood MB, MacKay SL, Rossi MJ, Schultz G. Five- minute treatments with fluorouracil, floxuridine, and mitomycin have long-term effects on human Tenon's capsule fibroblasts. *Arch Ophthalmol*1992;110:1150-54.
7. Wu KY, Hong SJ, Huang HT, Lin CP, Chen CW. Toxic effects of mitomycin-C on cultured corneal keratocytes and endothelial cells. *J Ocul Pharmacol Ther* 1999;15:401-11.
8. Derick RJ, Pasquale L, Quigley HA, Jampel H. Potential toxicity of mitomycin C. *Arch Ophthalmol*1991;109:1635.
9. Sihota R, Sharma T, Agarwal HC. Intraoperative mitomycin C and the corneal endothelium. *ActaOphthalmol Scand* 1998;76:80-82.
10. Tina T. L. Wong, Anna L. Mead, and Peng T. Khaw. Matrix Metalloproteinase Inhibition Modulates Postoperative Scarring after Experimental Glaucoma Filtration Surgery. *Investigative Ophthalmology and Visual Science*. 2003;44:1097-1103.
11. Grewal DS, Jain R, Kumar H, Grewal SP. Evaluation of subconjunctivalbevacizumab as an adjunct to trabeculectomy: a pilot study. *Ophthalmology* 2008;115:2141-45.
12. Cantor LB, Mantravadi A, WuDunn D *et al*. Morphologic classification of filtering blebs after glaucoma filtration surgery: the Indiana Bleb Appearance Grading Scale. *J Glaucoma* 2003;12:266-71.
13. Kahook MY, Schuman JS, Noecker RJ. Needle bleb revision of encapsulated filtering bleb with bevacizumab. *Ophthalmic Surg Lasers Imaging* 2006;37:148 -50.
14. 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.
15. Tuft SJ, Coster DJ. The corneal endothelium. *Eye* 1990;4:389- 424.
16. Joyce NC. Cell cycle status in human corneal endothelium. *ExpEye Res* 2005;81:629-38.
17. Pastor SA, Williams R, Hetherington J, Hoskins HD, Goodman D. Corneal endothelial cell loss following trabeculectomy with mitomycin C. *J Glaucoma* 1993;2:112-13.
18. Hosny MH, Zayed MA, Shalaby AM, Eissa IM. Effect of intracameralbevacizumab injection on corneal endothelial cells: an in vivo evaluation. *J OculPharmacolTher.* 2009;25:513–17.
19. PARK H.Y., KIM S.J., LEE H.B., *et al*.: Effect of intracameralbevacizumab injection on corneal endothelium in rabbits. *Cornea* 2008 27: 1151-55.
20. Guzel H, Bakbak B, Koylu MT, Gonul S, Ozturk B and Gedik S. The effect and safety of intravitreal injection of ranibizumab and bevacizumab on the corneal endothelium in the treatment of diabetic macular edema. *CutanOculToxicol* 2017;36:5-8.
21. Gagnon MM, Boisjoly HM, Brunette I, Charest M, Amyot M. Corneal endothelial cell density in glaucoma. Cornea 1997;16:314–18.
22. 22.`Arnavielle S, Lafontaine PO, Bidot S, Creuzot-Garcher C, D'Athis P, Bron AM. Corneal endothelial cell changes after trabeculectomy and deep sclerectomy. *J Glaucoma* 2007;16:324–28.
23. Okumura N, Matsumoto Daiki, Okazaki Y, Koizumi N, Sotozono C, Kinoshita S, *et al*. Wide-field contact specular microscopy analysis of corneal endothelium post trabeculectomy. *Graefes Arch Clin Exp Ophthalmol* 2018;256:1-8.

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