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TO STUDY THE EFFECT OF INTRASTROMAL VS SUBCONJUNCTIVAL ANTI VASCULAR ENDOTHELIAL GROWTH FACTOR AGENT BEVACIZUMAB IN CORNEAL NEOVASCULARIZATION"

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ABSTRACT

Background: Corneal neovascularization (CoNV) impairs visual function by disrupting corneal clarity, prompting the use of targeted anti-VEGF therapies. This study compares the clinical efficacy and safety of subconjunctival versus intrastromal administration of bevacizumab in managing CoNV. **Methods:** This prospective, interventional study enrolled 70 eyes diagnosed with CoNV, which were equally randomized into Group A (subconjunctival injection, n=35) and Group B (intrastromal injection, n=35). Day 28 outcomes were evaluated by measuring Best Corrected Visual Acuity (BCVA), monitoring for adverse events, and calculating a composite CoNV severity score based on involved quadrants, vessel density, and vessel length. **Results:** Both groups exhibited substantial vascular regression, with the mean total treatment score decreasing from 8.4 ± 2.03 at baseline to 6.42 ± 1.80 at Day 28 ($p < 0.001$). Group B demonstrated significantly better anatomical outcomes than Group A, achieving fewer vessels per quadrant (1.62 ± 0.54 vs 1.97 ± 0.74; $p = 0.032$) and a lower composite treatment score (6.0 ± 1.32 vs 6.85 ± 2.14; $p = 0.048$). Functionally, Group B achieved statistically significant BCVA improvement ($p = 0.0448$), whereas Group A's improvement was not statistically significant. Regarding safety, 14.3% of Group A experienced subconjunctival hemorrhage, while Group B reported zero adverse events. **Conclusion:** Both administration routes effectively regress CoNV, but intrastromal delivery provides superior structural regression, significant visual recovery, and fewer localized bleeding complications.

KEYWORDS: Corneal neovascularization, Bevacizumab, Intrastromal injection, Subconjunctival injection, Anti-VEGF

INTRODUCTION

The cornea's transparency and avascular nature are critical for optimal visual function. This clarity is maintained through precise collagen alignment, efficient endothelial pumping and a state of immune privilege [(1,2)] . Corneal neovascularization (CoNV) occurs when this delicate balance between angiogenic and anti-angiogenic factors is disrupted, leading to the abnormal invasion of new blood vessels into the typically avascular corneal stroma [(3)] . A variety of pathologies can trigger this process, including infections, ischemia, trauma, inflammation, chemical injuries and degenerative conditions. If left untreated, CoNV can result in severe structural and functional complications, such as lipid deposition, stromal scarring, corneal edema and an elevated risk of graft rejection following keratoplasty due to the infiltration of immune cells [(4,5)] .

A central molecular mediator in the pathogenesis of corneal angiogenesis is vascular endothelial growth factor (VEGF). VEGF promotes the proliferation of endothelial cells, increases vascular permeability and drives the formation of neovessels [(6)]. Recognizing the critical role of VEGF, anti-VEGF agents have emerged as promising targeted therapies for managing CoNV. Bevacizumab, a recombinant humanized monoclonal antibody that targets VEGF-A, was originally developed for retinal disorders but is increasingly being explored off-label for corneal pathologies, showing encouraging clinical outcomes [(7,8)].

The efficacy of bevacizumab in treating CoNV is significantly influenced by its route of administration, with topical, subconjunctival and intrastromal routes being the most investigated. Topical administration offers a non-invasive option but is often limited by poor corneal penetration due to the large molecular size of the bevacizumab molecule. Conversely, subconjunctival injections deliver the medication closer to the limbal vasculature, allowing for deeper corneal penetration, particularly in vascularized areas where the epithelial integrity is compromised [(9,10)]. Intrastromal administration involves depositing the drug directly into the corneal stroma. This route achieves a high local drug concentration and provides prolonged therapeutic exposure, making it an especially potent strategy for inducing the regression of deep and mature neovessels [(11)].

Despite the established use of anti-VEGF agents in ophthalmology, the optimal delivery method for CoNV remains a subject of clinical debate. Therefore, this study aims to comparatively evaluate the clinical efficacy and safety of subconjunctival versus intrastromal administration of bevacizumab in the management of corneal neovascularization. By assessing both anatomical and functional outcomes, this research seeks to contribute to the optimization of therapeutic strategies for this potentially sight-threatening condition.

MATERIALS AND METHODS

Study Design and Patient Selection

This was a prospective, interventional study conducted at the Department of Ophthalmology, Shyam Shah Medical College, and Sanjay Gandhi Memorial Hospital, Rewa (M.P.), between January 2024 to December 2024. The study included 70 eyes of 70 patients clinically diagnosed with corneal neovascularization (CoNV). Written informed consent was obtained from all participants prior to enrollment. Patients with active ocular infections (bacterial, fungal, or viral), severe corneal thinning, descemetocele, corneal perforation, or uncontrolled diabetes mellitus (or diabetic patients receiving anti-VEGF treatments) were excluded from the study.

Randomization and Blinding

The 70 enrolled eyes were randomized into two equal groups (n=35 per group) using an allocation concealment method. Pre-randomized group assignments were sealed in opaque envelopes. Group A received subconjunctival injections of bevacizumab, while Group B received intrastromal injections. The administering researcher was provided only the group code to maintain blinding of the specific treatment protocol.

Baseline Evaluation and Grading System

Detailed baseline ophthalmic examinations included measurement of Best Corrected Visual Acuity (BCVA) converted to LogMAR equivalents. Slit-lamp biomicroscopy was performed to assess limbal ischemia, corneal clarity, epithelial integrity, stromal findings, and endothelial changes. Intraocular pressure (IOP) was measured via non-contact tonometry (NCT).

CoNV severity was documented photographically and graded using a modified semi-quantitative scale ranging from 0 to 4 across three parameters:

- Number of Involved Quadrants: Scored from 1 (minimal, 1 quadrant) to 4 (extensive, 4 quadrants).
- Vessel Density: Scored from 1 (1–5 vessels/quadrant) to 4 (>15 vessels/quadrant).
- Vessel Length: Scored based on proportionate length from limbus: 1 (peripheral <2mm), 2 (peripheral >2mm), 3 (reaching central cornea), 4 (involving central cornea with fibrosis).

A total pre-treatment score (ranging from 3 to 12) was calculated by summing the individual scores.

Intervention Protocol

Bevacizumab (100mg/4ml vial) was prepared under sterile conditions using a 1 mL tuberculin syringe. A 0.1 mL dose containing 2.5 mg of bevacizumab was administered.

- Group A (Subconjunctival): Under topical anesthesia, 0.1 mL of bevacizumab was injected subconjunctivally using a 27G needle near the affected quadrant, creating a visible bleb while avoiding scleral perforation.
- Group B (Intrastromal): Using a 27G needle (bevel down), the injection was introduced obliquely from the uninvolved area into the mid-stromal level. Corneal hydration guided the injection extent, followed by slight plunger withdrawal to prevent drug back-leakage.

Post-injection, patients were observed for 15–30 minutes and prescribed topical antibiotics (e.g., moxifloxacin) for 7 days.

Follow-up and Outcome Measures

Patients were reviewed on Day 7 to monitor for delayed adverse effects, with the final comprehensive evaluation conducted on Day 28. Day 28 assessments included repeat BCVA, IOP measurement, slit-lamp biomicroscopy, and CoNV grading via photography.

Outcomes were categorized as:

- Good Outcome: ≥50% regression in CoNV length and density, ≥2 lines improvement in visual acuity, and absence of adverse effects.
- Poor Outcome: ≤25% CoNV regression, lack of visual acuity improvement (or worsening), and presence of adverse side effects.

Statistical Analysis

Data were processed using Microsoft Excel and analyzed with SPSS software (version 20). Qualitative variables were analyzed using the Chi-square test, while quantitative data were assessed via paired and unpaired t-tests. A p-value of <0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

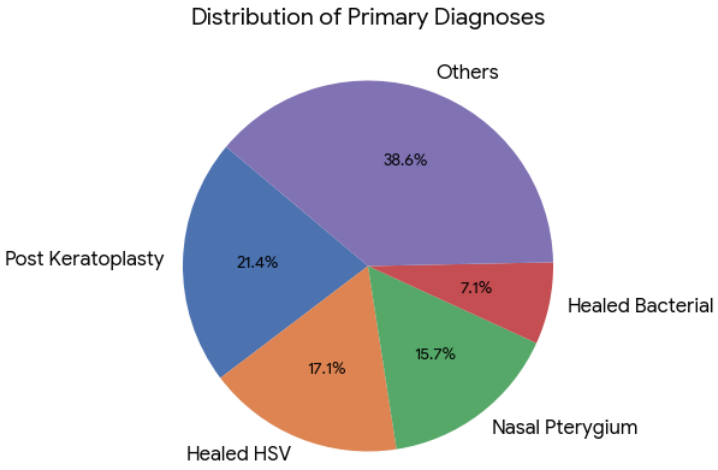
1. Baseline Demographics and Clinical Characteristics

A total of 70 eyes of 70 patients were included in the study, evenly randomized into two groups: Group A (Subconjunctival Bevacizumab, n=35) and Group B (Intrastromal Bevacizumab, n=35). The mean age of the study

population was 54.36 ± 19.25 years. The demographic distribution demonstrated a male predominance, with males comprising 57.15% in Group A and 65.72% in Group B.

Parameter	Group A (n=35)	Group B (n=35)	Total (n=70)
Male, n (%)	20 (57.15%)	23 (65.72%)	43 (61.4%)
Female, n (%)	15 (42.85%)	12 (34.28%)	27 (38.6%)

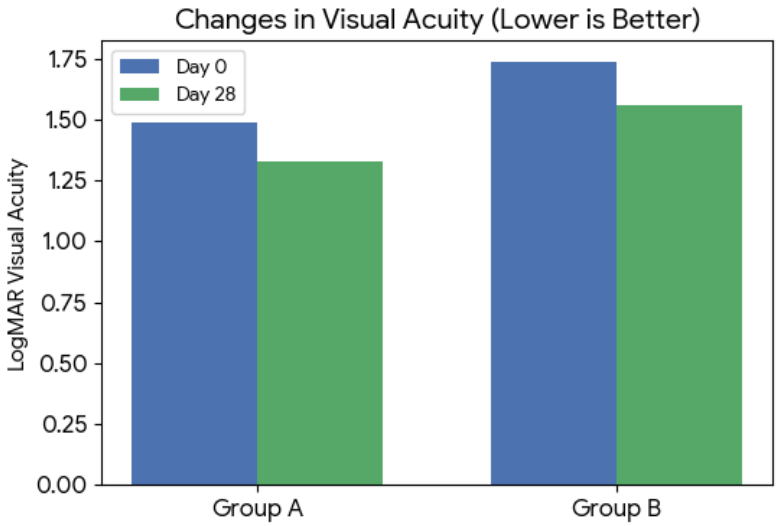
The most frequent primary diagnosis among the cohort was status post-keratoplasty (21.4%), followed by healed HSV keratitis (17.1%) and nasal pterygium (15.7%)



2. Visual Acuity Outcomes

Overall visual acuity (LogMAR) improved significantly from 1.61 ± 0.50 at baseline to 1.44 ± 0.53 at Day 28 ($p < 0.001$). Subgroup analysis revealed that Group B experienced a statistically significant visual improvement ($p = 0.0448$), whereas the change in Group A did not reach statistical significance ($p = 0.080$).

Group	Day 0 (Mean ± SD)	Day 28 (Mean ± SD)	P-value
Group A (Subconjunctival)	1.49 ± 0.52	1.33 ± 0.53	0.080
Group B (Intrastromal)	1.74 ± 0.49	1.56 ± 0.53	0.0448
Overall	1.61 ± 0.50	1.44 ± 0.53	< 0.001



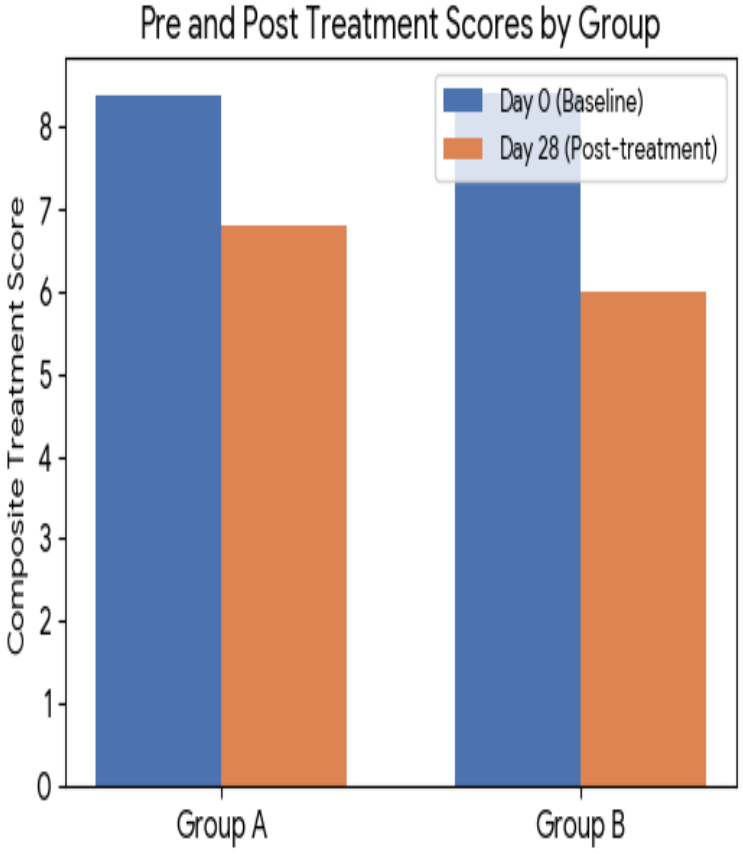
3. Changes in Corneal Neovascularization Parameters

Both treatment modalities yielded substantial regression in corneal neovascularization parameters. The overall treatment score across the population reduced from 8.4 ± 2.03 to 6.42 ± 1.80 ($p < 0.001$).

3.1 Comparison of Treatment Scores

Parameter	Group A (Day 0)	Group A (Day 28)	P-value	Group B (Day 0)	Group B (Day 28)	P-value

Involved Quadrants	2.8 ± 0.86	2.2 ± 1.07	0.003	2.85 ± 1.14	1.97 ± 0.74	<0.001
Vessels per Quadrant	2.6 ± 0.91	1.97 ± 0.74	<0.001	2.57 ± 0.88	1.62 ± 0.54	<0.001
Proportionate Length	2.97 ± 1.07	2.6 ± 0.97	<0.001	3.00 ± 1.05	2.4 ± 0.81	<0.001
Treatment Score	8.37 ± 1.94	6.8 ± 2.14	<0.001	8.42 ± 2.18	6.0 ± 1.32	<0.001



Inter-group comparison at Day 28 revealed that Group B had a significantly lower number of corneal vessels per quadrant (1.62 ± 0.54 vs 1.97 ± 0.74; p = 0.032) and a significantly better composite treatment score (6.0 ± 1.32 vs 6.85 ± 2.14; p = 0.048) compared to Group A, suggesting superior anatomical regression with intrastromal delivery.

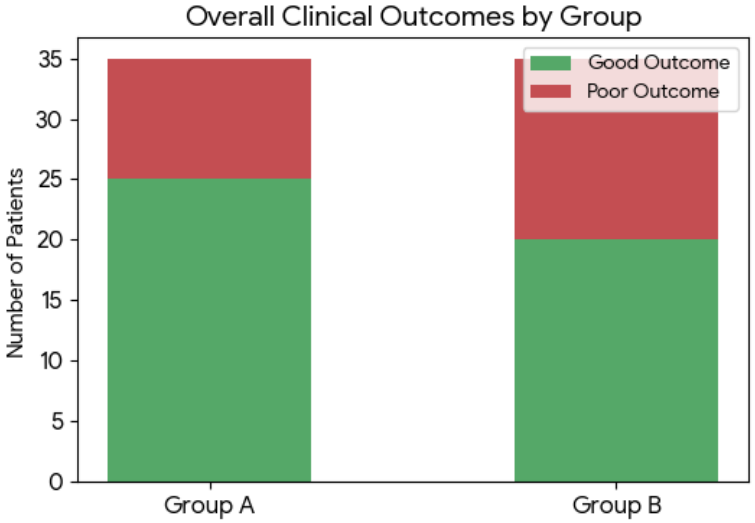
4. Safety and Clinical Outcomes

Adverse events were limited and strictly localized. Subconjunctival hemorrhage occurred in 5 patients (14.3%) in Group A, whereas none were reported in Group B . No severe complications such as globe perforation or corneal thinning were observed in either cohort.

Adverse Reaction	Group A	Group B
Subconjunctival hemorrhage	5	0
Corneal thinning	0	0
Anterior chamber reaction	0	0
Globe perforation	0	0

Regarding the overall clinical outcome, a 'Good' outcome (defined as ≥50% vascular regression, ≥2 lines VA improvement, and no adverse effects) was achieved by 64.3% of the study population. Group A had a higher absolute number of good outcomes (25 vs 20), but Group B was unburdened by adverse events.

Outcome Measure	Group A	Group B	Total
Good	25 (71.4%)	20 (57.1%)	45 (64.3%)
Poor	10 (28.6%)	15 (42.9%)	25 (35.7%)



DISCUSSION

Corneal neovascularization (CoNV) poses a significant threat to corneal transparency and visual acuity by compromising the structural clarity and immune privilege of the eye. The role of vascular endothelial growth factor (VEGF) in mediating corneal angiogenesis is well-established, making anti-VEGF agents like bevacizumab a cornerstone of modern targeted pharmacotherapy. However, the optimal route of drug delivery remains a critical subject of clinical debate. This prospective, interventional study evaluated the comparative efficacy and safety of intrastromal (IS) versus subconjunctival (SC) administration of bevacizumab in 70 eyes with CoNV.

The demographic profile of our cohort demonstrated a mean age of 54.36 ± 19.25 years with a male predominance (61.4%), which aligns closely with previous demographic data reported by Bahar et al. [12] and Petsoglou et al. [13]. The most prevalent underlying etiologies for CoNV in our study were status post-keratoplasty (21.4%), healed HSV keratitis (17.1%), and pterygium (15.7%). This distribution mirrors the findings of Doctor et al. [14] and Vieira et al.[15], highlighting surgical interventions and herpetic infections as high-risk triggers for aggressive neovascularization.

Overall, our study demonstrated that both SC and IS routes are highly effective in regressing CoNV. The overall treatment score improved markedly from 8.4 ± 2.03 to 6.42 ± 1.80 ($p < 0.001$) over a 28-day period. In the subconjunctival group (Group A), we noted a statistically significant reduction in the number of involved quadrants, vessel density, and proportionate vessel length ($p < 0.001$). This robust anatomical response corroborates the findings of You et al. [16] and Zaki et al.[17], who reported substantial short-term regression of superficial CoNV and minor vessels following SC bevacizumab. Similarly, Krizova et al.[18] demonstrated a significant decrease in the extent of peripheral and central CoNV segments following SC delivery.

Despite the strong anatomical response in the SC group, the improvement in mean best-corrected visual acuity (BCVA) (1.49 to 1.33 LogMAR) did not reach statistical significance ($p = 0.080$). This observation is consistent with Bahar et al., who reported that while SC bevacizumab effectively achieved partial vessel regression, it did not always translate into meaningful short-term visual improvement. This suggests that superficial regression alone may be insufficient to fully restore central visual function, especially when deeper stromal haziness or lipid deposition persists.

Conversely, the intrastromal group (Group B) exhibited profound improvements across both anatomical and functional parameters. The highly significant reductions ($p < 0.001$) in vessel density, length, and involved quadrants strongly support the efficacy of IS delivery. These results parallel the findings of Sarah et al. [19] who observed complete regression of vascularization in a majority of treated cases, and Gupta et al., who highlighted the long-term effectiveness of IS bevacizumab in managing chronic deep CoNV. By depositing the medication directly into the mid-stroma, IS administration achieves a higher, sustained local drug concentration, effectively targeting mature, deep-seated neovessels.

A pivotal finding of our study is the comparative superiority of the IS route over the SC route. At Day 28, Group B demonstrated a significantly lower mean number of corneal vessels per quadrant (1.62 ± 0.54 vs. 1.97 ± 0.74, $p = 0.032$) and a superior composite treatment score (6.0 ± 1.32 vs. 6.85 ± 2.14, $p = 0.048$) compared to Group A. This clinical superiority is strongly validated by the experimental research conducted by Ucgul et al.[20], which proved that intrastromal delivery of anti-VEGF agents yielded a significantly greater reduction in CoNV area in animal models compared to subconjunctival injections.

Functionally, the IS group achieved a statistically significant improvement in BCVA ($p = 0.0448$). This enhanced visual recovery aligns with findings by Vieira et al. and Hashemian et al.[21], suggesting that IS administration better facilitates the clearance of deep stromal vessels encroaching on the optical axis, thereby effectively reducing associated corneal edema and lipid exudation.

Regarding safety, both modalities were well-tolerated with no instances of severe complications such as globe perforation or corneal thinning. However, 14.3% of patients in the SC group developed localized subconjunctival hemorrhage, a well-documented and self-limiting consequence of this injection technique. No such localized bleeding was observed in the IS group. While the IS route completely circumvented conjunctival bleeding and showed a superior side-effect profile in this cohort, it inherently demands greater technical precision and microscopic control to avoid inadvertent intraocular penetration.

In conclusion, while both subconjunctival and intrastromal bevacizumab injections are safe and effective treatments for corneal neovascularization, the intrastromal route offers superior therapeutic outcomes. It provides greater regression of vessel density, more significant functional visual recovery, and a lower incidence of localized bleeding complications. Intrastromal delivery should be strongly considered as the preferred approach, particularly in cases of deep or visually threatening neovascularization.

CONCLUSION

This prospective, interventional study demonstrates that while both subconjunctival and intrastromal injections of bevacizumab are effective and safe for treating corneal neovascularization (CoNV), the intrastromal route offers statistically superior therapeutic outcomes. Specifically, intrastromal delivery results in more profound regression of vessel density, significantly greater functional visual recovery (BCVA), and complete avoidance of the localized subconjunctival hemorrhages associated with the subconjunctival approach. By achieving a higher local drug concentration directly at the site of deep-seated neovessels, intrastromal administration maximizes the anti-angiogenic effects of bevacizumab. Consequently, intrastromal delivery should be considered the preferred modality for managing deep and visually threatening CoNV, provided the operating physician possesses the requisite microsurgical expertise to safely perform the procedure.

LIMITATIONS

Several limitations of the present study warrant consideration. First, the relatively small sample size of 70 eyes and the short-term follow-up duration (28 days) restrict our ability to definitively evaluate long-term efficacy, recurrence rates, and the potential need for repeat injections. Second, the study lacked a true placebo or natural history control group, as it was designed to compare two active therapeutic routes. Third, being a single-center study, the demographic and etiological profile of the cohort may not be entirely generalizable to broader, more diverse populations. Additionally, while structural improvements were objectively scored, the reliance on slit-lamp photography rather than advanced quantitative imaging modalities, such as Anterior Segment Optical Coherence Tomography Angiography (AS-OCTA), may limit the precision of deep vessel analysis. Future multicenter, randomized controlled trials with extended follow-up periods and high-resolution objective imaging are necessary to validate these findings and establish standardized, evidence-based dosing protocols.

REFERENCES

1. Shakiba Y, Mansouri K, Arshadi D, Rezaei N. Corneal neovascularization: molecular events and therapeutic options. Recent Pat Inflamm Allergy Drug Discov. 2009 Nov;3(3):221-31.
2. Rates ERD, Almeida CD, Costa EdPF, Farias RJdM, Santos-Oliveira R, Alencar LMR. Layer-by-Layer Investigation of Ultrastructures and Biomechanics of Human Cornea. Int J Mol Sci. 2022;23(14):7833.
3. Hos D, Bukowiecki A, Horstmann J, et al. Transient Ingrowth of Lymphatic Vessels into the Physiologically Avascular Cornea Regulates Corneal Edema and Transparency. Sci Rep. 2017;7:7227.
4. Sharif Z, Sharif W. Corneal neovascularization: updates on pathophysiology, investigations & management. Rom J Ophthalmol. 2019 Jan-Mar;63(1):15-22.
5. Dastjerdi MH, Al-Arfaj KM, Nallasamy N, et al. Topical bevacizumab in the treatment of corneal neovascularization. Arch Ophthalmol. 2009 Apr;127(4):381-9.
6. Mirabelli P, Mukwaya A, Lennikov A, et al. Genome-wide expression differences in anti-Vegf and dexamethasone treatment of inflammatory angiogenesis in the rat cornea. Sci Rep. 2017;7:7616.
7. Hirose M, Matsumiya W, Honda S, Nakamura M. Efficacy and visual prognostic factors of intravitreal bevacizumab for macular edema. Clin Ophthalmol. 2014;8:2301-2305.
8. Bhatti N, Qidwai U, Hussain M, Kazi A. Efficacy of topical bevacizumab in high-risk corneal transplant survival. Pak J Med Sci. 2013 Apr;29(2):519-22.
9. Lodhi AA, Murtaza, Ahmed M, et al. Role of tetracycline in corneal neovascularization. Pak J Ophthalmol. 2015;31:150-3.
10. Gupta AA, Mammo DA. Intrastromal Bevacizumab in the management of corneal neovascularization: a retrospective review. Graefes Arch Clin Exp Ophthalmol. 2020;258(1):167-173.
11. Kim RY, Chung SK, Kim MS, Ra H. Effects of combined photodynamic therapy and topical bevacizumab treatment on corneal neovascularization in rabbits. Cornea. 2016;35:1615-20.
12. Bahar I, Kaiserman I, McAllum P, et al. Subconjunctival bevacizumab injection for corneal neovascularization. Cornea. 2008;27(2):142-147.
13. Petsoglou C, Rayner S, Conway RM, et al. Subconjunctival bevacizumab induces regression of corneal neovascularisation: a pilot randomised placebo-controlled double-masked trial. Br J Ophthalmol. 2013;97(1):28-32.

14. Doctor PP, Bhat PV, Foster CS. Subconjunctival bevacizumab for corneal neovascularization. Cornea. 2008;27(9):992-995.
15. Vieira AC, Pereira T, de Freitas D. Intrastromal injection of bevacizumab in patients with corneal neovascularization. Am J Ophthalmol. 2012;153(4):707-714.
16. You IC, Ahn M, Ryu SW, et al. Treatment of corneal neovascularization with subconjunctival and intrastromal injections of bevacizumab. Cornea. 2009;28(9):1002-1006.
17. Zaki AA, Farid SF. Subconjunctival bevacizumab for corneal neovascularization. Acta Ophthalmol. 2010;88(8):868-871.
18. Krizova D, Vokrojova M, Liehneova I, et al. Treatment of corneal neovascularization using anti-VEGF bevacizumab. J Ophthalmol. 2014;2014:178132.
19. Sarah S, et al. Effectiveness of intrastromal bevacizumab injections in treating corneal neovascularization. Cornea. 2016.
20. Ucgul AY, et al. Comparison of subconjunctival and intrastromal administration of anti-VEGF agents on suture-induced corneal neovascularization. Exp Eye Res. 2021.
21. Hashemian MN, Zare MA, Rahimi F, et al. Deep intrastromal bevacizumab injection for management of corneal stromal vascularization after deep anterior lamellar keratoplasty. Cornea. 2009;28(9):1078-1079.