Effect of repeated intravitreal bevacizumab on corneal parameters in age-related macular degeneration

Hakan Koc[1], *Ugur Bayram*[1], *Serkan Ozen*[1]

ABSTRACT

Purpose: To evaluate effect of three consecutive doses of intravitreal bevacizumab (IVB) injection on corneal parameters in exudative type age related macular degeneration (ARMD).

Materials and Method: A total of 50 eyes of 50 patients, who were diagnosed with exudative ARMD after the examinations and were administered three doses of 1.25 mg/0.05 ml IVB one month apart, were evaluated prospectively. Specular microscopy was performed to evaluate central corneal thickness, endothelial cell count, percentage of hexagonal cells, and coefficient of variation. Corneal structural parameters measured before IVB and after three doses of IVB were compared.

Results: The average age of the patients participating in the study was 75.08 ± 4.9 years. Statistically significant increase was observed in the central corneal thickness before injection compared to the value after three doses of injection ($p = 0.004$). While the cell density of the patients was 2406.34 ±2 12.28 (cells/mm²) before the injection, cell density was measured as 2388.70 ± 208.88 (cells/mm²) after three doses of injection. There was no statistically significant increase in pre-injection CV percentage compared to the three dose post-injection value ($p = 0.06$). While the average HEX percentage of patients before the injection was 45.80 ± 4.83 (%),average HEX percentage after injections was measured at 44.66 ± 4.27 (%). The difference between these two measurements was statistically significant ($p = 0.03$).

Conclusion: With aging, tight junctions in corneal epithelial and endothelial cells may become more sensitive. IVB injections administered in three consecutive doses may cause changes in corneal endothelial cell morphology by causing sensitized tight junctions to be damaged more easily.

Keywords: Exudative type age-related macular degeneration, Intravitreal bevacizumab, Corneal endothelial cell, Specular microscopy.

INTRODUCTION

Age-related macular degeneration (ARMD) is one of the most common causes of permanent visual impairment and blindness among people over the age of 60 in developed countries. By 2040, the number of people with ARMD worldwide is projected to be 288 million.[1]

Intravitreal administration of vascular endothelial growth factor antagonists (anti-VEGF) is considered the gold standard for the treatment of exudative ARMD.[2] Bevacizumab (Avastin, Roche-Genentech Inc., South San Francisco, CA) is a full-length humanized monoclonal IgG antibody that binds to all isoforms of VEGF. In 2004, it was approved by the US Food and Drug Administration as the first antiangiogenic agent for the treatment of metastatic colorectal cancer.[3] After intravitreal administration and significant results in VEGF-mediated diseases such as choroidal neovascularization[4], central retinal vein occlusion[5], and proliferative diabetic retinopathy[6], it has been used off-label in ophthalmology.

After intravitreal injection of bevacizumab into rabbit eyes, bevacizumab was detected in the aqueous humor of both injected and non-injected eyes. It was also found to persist in the injected eye for more than a month.[7] In addition, VEGF and its receptors have been shown to be

1- Department of Ophthalmology, Giresun University Faculty of Medicine, Giresun, Türkiye

Received: 24.04.2024
Accepted: 13.09.2024
J Ret-Vit 2024; 33: 245-250
DOI:10.37845/ret.vit.2024.33.40

Correspondence author:
Hakan Koc
Email: hakankoc9@hotmail.com

present in the corneal endothelium.[8] Considering all these results, potential corneal cytotoxicity is of concern. Bayar et al. reported that intravitreal injection of bevacizumab may cause corneal changes and that the safety and corneal effects of bevacizumab should be evaluated in detail.[9] This study was designed to prospectively evaluate the effect of intravitreal bevacizumab (IVB) on corneal endothelial parameters in exudative age-related macular degeneration with a one-month interval.

MATERIALS AND METHODS

Ethics committee approval was obtained from the Local Ethics Committee of Giresun Training and Research Hospital for this study (IRB 13.03.2023/10). Informed consent was obtained from all participants before inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki. Fifty eyes of 50 patients with newly diagnosed exudative age-related macular degeneration (ARMD) admitted to the Ophthalmology Clinic of the Training and Research Hospital were included in the single-center prospective study. All eyes underwent a complete ophthalmologic examination, including refraction measurements with an autorefractometer (Topcon Auto Ref-Keratometer, Tokyo, Japan), best corrected visual acuity (BCVA) assessment, intraocular pressure measurements, slit lamp examination, slit lamp biomicroscopic evaluation, Optical Coherence Tomography (OCT), and Fundus Fluorescein Angiography (FFA). Considering that outcome measurements obtained from both eyes of the same subject tend to be positively correlated, the right eye was selected for study measurements if both eyes were eligible for inclusion. Patients who were diagnosed with exudative age-related macular degeneration after the examinations and who received three doses of 1.25 mg/0.05 ml IVB at one-month intervals were included in the study.

Exclusion Criteria:

- History of ocular surgery
- Previous intravitreal injection
- Corneal endothelial cell count below 1000/mm^2 before injections
- History of ocular trauma
- Have had any ocular surgery between injections or in the three months prior to the first injection
- Keratitis or corneal degeneration
- On antiglaucomatous medication
- Use of drugs with known corneal toxicity
- Contact lens wear or systemic disease that may affect the ocular surface

Injection Technique

Topical anesthesia with proparacaine (Alcaine 0.5%; Alcon AG, Geneva, Switzerland) was applied before all injections. The eyelid and periocular surface were disinfected with 10% povidone iodine and covered with an ophthalmic drape. Then, 5% povidone iodine was instilled into the cornea, conjunctiva, and palpebral fornix and left for three minutes and washed with a balanced sterile saline solution. Bevacizumab was injected intravitreally into the superotemporal quadrant at a distance of 3.5 mm from the limbus with a 30 gauge needle. After the injection, prophylactic topical 0.5% Moxifloxacin (Moxai; Abdi İbrahim İlaç ve Sanayi, Istanbul, Turkey) was prescribed five times a day for five days. Biomicroscopic examination and intraocular pressure measurement were performed on the first day after injection.

Corneal Endothelial Morphology Analysis

Endothelial cells were assessed using the auto-analysis mode of a non-contact specular microscopy (SP2000P; Topcon Corp., Tokyo, Japan) instrument performed by the same ophthalmologist. CD: Endothelial Cell Density (mm^2), Min: Minimum Cell Area (μm^2), N: Normal Cell Area (μm^2), Max: Maximum Cell Area (μm^2), Mean: Mean Cell Area (μm^2), SD: Mean Standard Deviation of Cell Area (μm^2), CV: Coefficient of Variation: SD divided by AVG (mean cell area), CD: number of cells per mm^2, HEX: hexagonality ratio (%), and CCT: central corneal thickness. To evaluate the effect of bevacizumab on the corneal endothelium, measurements were taken by specular microscopy before injection and after three consecutive injections of 1.25 mg/0.05 ml bevacizumab, repeated one month apart. Endothelial number and morphology were evaluated by specular microscopy before injection and at one week after three doses of IVB injection.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 for Windows (IBM Corp., Armonk, NY, USA). Paired sample t test and Wilcoxon test were used to analyze quantitative dependent data. A probability level of $P<0.05$ was considered statistically significant.

RESULTS

The mean age of the 50 patients included in our study was 75.08 ± 4.9 years. 23 of the patients were male and 27 were female. The central corneal thickness of the patients was 540.46

± 25.16 µm before the injections and 548.50 ± 24.89 µm after the injections. The difference between these two measurements was statistically significant (p = 0.004).

The endothelial cell density of the patients was 2406.34 ± 212.28 (cells/mm²) before the injections and 2388.70 ± 208.88 (cells/mm²) after the injections. The difference between these two measurements was statistically significant (p = 0.001). The mean coefficient of variation (CV) percentage before injections was 40.10 ± 7.45, while the mean CV percentage after injections was 41.40 ± 5.84. We observed no statistically significant difference between these two measurements (p = 0.06).

The mean HEX percentage before the injections was 45.80 ± 4.83, while the mean HEX percentage after the injections was 44.66 ± 4.27. The difference between these two measurements was statistically significant (p = 0.03). The mean N measurement before injections was 175.36 ± 27.97, while the mean N measurement after injections was 179.08 ± 30.907. No statistically significant difference was observed between these two measurements (p = 0.201).

The mean MIN values of the patients were 178.46 ± 18.22 µm² before the injections and 184.84 ± 24.54 µm² after the injections. No statistically significant difference was observed between these two measurements (p = 0.105). The mean MAX value before injections was 906.96 ± 73.21 µm², while the mean MAX value after injections was 914.72 ± 60.39 µm². The difference between these two measurements was statistically significant (p = 0.040).

The mean AVG values of the patients were 397.54 ± 29.50 µm² before the injections and 403.20 ± 30.16 µm² after the injections. The difference between these two measurements was statistically significant (p = 0.001). The mean SD before the injections was 136.58 ± 16.13 µm², while the mean SD after the injections was 138.44 ± 15.10 µm². No statistically significant difference was observed between these two measurements (p = 0.12). The data obtained from

Table 1: *Effects of Intravitreal Bevacizumab Injection on CD, CoV, Hex, CCT, AVG, N, Min, Max and SD Values*

Parameters	Pre-injectiion (mean±SD)	Post-injection (mean±SD)	p value
Central Corneal Thickness (µm)	540,46 ± 25,16	548,50 ± 24,89	**0,004**
Cell Density (cells/mm²)	2406,34 ± 212,28	2388,70 ± 208,88	**0,001**
Coefficient variation (%)	40,10 ± 7,45	41,40 ±5,84	0,06
Hexagonality (%)	45,80 ± 4,83	44,66 ±4,27	**0,030**
N (cells)	175,36±27,97	179,08±30,907	0,201
Min (µm²)	178,46±18,22	184,84±24,54	0,105
Max (µm²)	906,96±73,21	914,72±60,39	**0,040**
Avg (µm²)	397,54±29,50	403,20±30,16	**0,001**
SD (µm²)	136,58±16,13	138,44±15,10	0,120

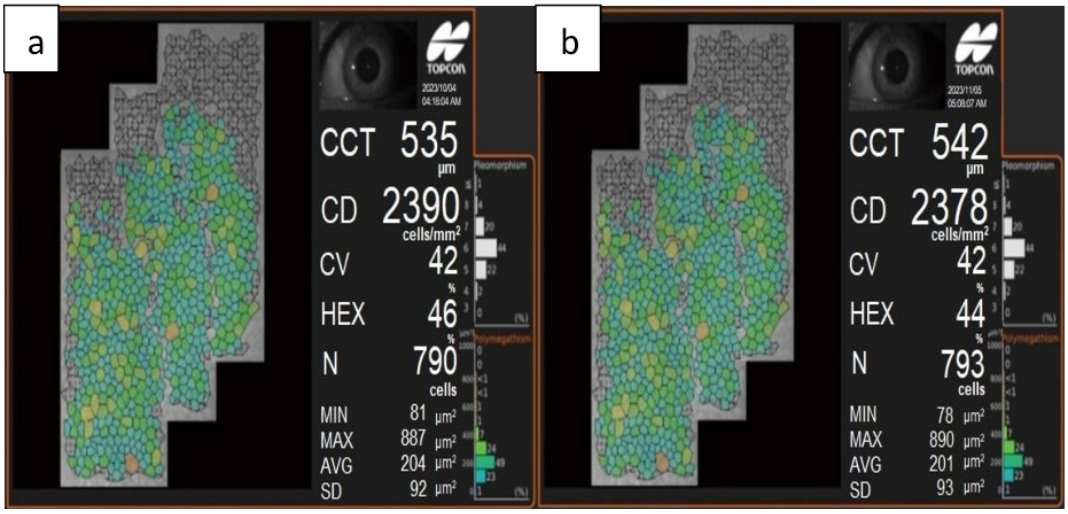

Figure 1: *Specular microscopy results before intravitreal injection (a) and after three doses of intravitreal bevacizumab treatment (b).*

the study are summarized in Table 1. Specular microscopy measurements of one patient before and after three doses of IVB injection are shown in Figure 1.

After intravitreal injections, there was no intraocular pressure increase requiring antiglaucomatous treatment.

DISCUSSION

The standard treatment for neovascular age-related macular degeneration (ARMD) is inhibitors against vascular endothelial growth factor (VEGF), which plays an important role in the pathogenesis of the disease.[10] Bevacizumab (Avastin, Genentech, South San Francisco, CA) is a recombinant humanized monoclonal immunoglobulin antibody that inhibits human vascular endothelial growth factor and is administered intravitreally for the treatment of VEGF-mediated diseases such as choroidal neovascularization.[4] In our country, in patients with exudative age-related macular degeneration who are scheduled for intravitreal injection therapy, the initial IVB loading dose must be administered in three consecutive doses (1.25 mg/0.05 ml) 4-6 weeks apart. The corneal endothelium plays a crucial role in maintaining corneal clarity due to its pump function.[11] Vascular endothelial growth factor and its receptors have been shown to be present in endothelial cells contributing to corneal transparency.[12] Gharbiya et al. reported that intracameral and intravitreal anti-VEGFs (Ranibizumab, Aflibercept) administered to rabbits may induce apoptosis in the corneal endothelium in intracameral administration.[13] Akal et al. showed that a high level of apoptotic activity was observed in the corneal endothelium after intracameral bevacizumab administration to rats.[14] These studies suggest that the safety of repeated dose intravitreal anti-VEGF injections should be investigated. Although there are studies in the literature examining the effect of intravitreal anti-VEGF administration on corneal endothelium, we could not find any studies examining the effect of repeated IVB administration on corneal endothelium in patients with isolated exudative type age-related macular degeneration. Chiang et al. evaluated endothelial cell density on first day, first week, third month, and sixth month after a single-dose IVB injection and reported that there was no significant change compared to the pre-injection period.[15] In contrast to our study, the patient groups were heterogeneous in this study, and the effect of single dose IVB on corneal endothelial morphology was evaluated. Horozoğlu et al. reported that intravitreal administration of a single dose of 1.25 mg/0.05 ml bevacizumab or 0.5 mg/0.05 ml ranibizumab to patients with DME or age-related macular degeneration did not cause a significant change in corneal endothelial count at first week and first month after treatment. In addition, the heterogeneity of the patients, such as DME and neovascular age-related macular degeneration was shown as a limitation of the study.[16] Park et al. evaluated corneal endothelial morphology before injection and at the second, fifth, and 30th minute after injection to assess endothelial changes that may be related to elevated intraocular pressure in the acute period after IVB and reported that no changes were observed in corneal endothelial morphology and central corneal thickness.[17] Ulutas et al. examined corneal endothelial parameters along with anterior chamber parameters in patients who received at least three doses of IVB for any diagnosis and reported that no statistically significant changes were observed in corneal endothelial parameters and central corneal thickness after IVB injections.[18] Guzel et al. reported that there was no difference in corneal endothelial cell parameters before, at one month, and at three months after three consecutive injections of 0.5 mg/0.05 ml IVB or 1.25 mg/0.05 ml intravitreal Ranibizumab for DME.[19] It is important that the intravitreal injections in this study were performed for a single disease.

In contrast to these studies, Lee et al. reported a decrease in endothelial cell number and hexagonality and an increase in CCT in Diabetes Mellitus (DM) lasting longer than 10 years.[20] Durukan et al. reported that in patients with type 2 DM, corneal endothelial cell density decreased, morphology deteriorated, and corneal thickness increased, and this deterioration was highest in proliferative diabetic retinopathy, and there was a correlation between the stage of diabetic retinopathy and corneal endothelial changes.[21] In the study reported by Guzel et al.[19], the duration of DM disease was reported as 11.90 ± 5.04 years in the bevacizumab group and 14.10 ± 6.32 years in the Ranibizumab group, and they were not divided into groups according to disease duration. In addition, considering that corneal endothelial parameters may change according to the stage of diabetic retinopathy, the patients in the study were not classified according to the degree of diabetic retinopathy, so the patients in the study included a partially heterogeneous group. Arslan et al. evaluated anterior chamber parameters at the first and second month after two consecutive doses of the same anti-VEGF (Ranibizumab, Aflibercept, and Bevacizumab) for any disease and reported no change in central corneal thickness but a decrease in corneal endothelial cell density.[22] The fact that the groups in this study were not classified according to

the intravitreal drugs administered does not provide clear information about which drug caused this change. Joshi et al. reported no change in corneal endothelial count at one month after intravitreal injection. In this study, the disease groups were age-related macular degeneration and retinal vascular occlusion and included heterogeneous groups.[23]

When only the effects of intravitreal injection on central corneal thickness were examined, Gumuş et al. evaluated intraocular pressure and central corneal thickness at first hour and 24th hours after a single dose of IVB injection for any disease and reported that central corneal thickness increased in the early period (first hour) due to increased intraocular pressure, but IOP and CCT were similar to pre-injection values at 24th hours.[24] Another study evaluated central corneal thickness on the third day, 15th day, and first month after a single-dose IVB injection for any disease and showed that no difference was observed compared to the pre-injection period.[25] In our study, we found that IVB injections administered at three doses at one month intervals increased central corneal thickness and decreased corneal endothelial cell count and hexoganality in exudative type age-related macular degeneration. The reason for this difference may be due to the advanced age of the patient group since the patient group had only age-related macular degeneration. There are in vitro studies showing that anti-VEGF agents can disrupt the tight junctions of the RPE.[26] Especially with aging, the tight junctions in corneal epithelial and endothelial cells may become more sensitive.[27] The tight junctions that become sensitive may be more easily damaged by intravitreal anti VEGF injections. By this mechanism, we think that IVB administration in three consecutive doses may affect endothelial cell morphology by disrupting the tight junctions of the corneal endothelium. As stated by Raghuram et al., VEGFs, which have the ability to diffuse, pass from the vitreous to the anterior segment due to concentration differences and are cleared by the trabecular meshwork.[28] We think that repeated IVB injections may pass into the anterior chamber and have a toxic effect on corneal endothelial cells, especially in elderly patients, and therefore may cause a decrease in corneal endothelial cell number.

The main limitations of our study are the small sample size and short follow-up period. In addition, we only evaluated the results of three consecutive bevacizumab injections.

In conclusion, this is the first study to evaluate the effect of three consecutive bevacizumab injections on corneal parameters in patients with age-related macular degeneration. We recommend caution when performing repeated IVB injections after three consecutive IVB injections in patients with age-related macular degeneration due to the age-related effects of intravitreal anti-VEGF on corneal tight junctions.

REFERENCES

1. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health 2014;2:e106-e116. https://doi.org/10.1016/S2214-109X(13)70145-1
2. Lotery A, Griner R, Ferreira A, Milnes F, Dugel P. Real-world visual acuity outcomes between ranibizumab and aflibercept in treatment of neovascular AMD in a large US data set. Eye (Lond) 2017;31:1697-1706. https://doi.org/10.1038/eye.2017.143
3. Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochem Biophys Res Commun 2005;333:328-335. https://doi.org/10.1016/j.bbrc.2005.05.132
4. Rich RM, Rosenfeld PJ, Puliafito CA, et al. Short-term safety and efficacy of intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Retina 2006;26:495-511. https://doi.org/10.1097/01.iae.0000225766.75009.3a
5. Iturralde D, Spaide RF, Meyerle CB, et al. Intravitreal bevacizumab (Avastin) treatment of macular edema in central retinal vein occlusion: a short-term study. Retina 2006;26:279-284. https://doi.org/10.1097/00006982-200603000-00005
6. Spaide RF, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. Retina 2006;26:275-278. https://doi.org/10.1097/00006982-200603000-00004
7. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 2007;114:855-859. https://doi.org/10.1016/j.ophtha.2007.01.017
8. Gan L, Fagerholm P, Palmblad J. Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in the regulation of corneal neovascularization and wound healing. Acta Ophthalmol Scand 2004;82:557-563. https://doi.org/10.1111/j.1600-0420.2004.00312.x
9. Bayar SA, Altinors DD, Kucukerdonmez C, Akova YA. Severe corneal changes following intravitreal injection of bevacizumab. Ocul Immunol Inflamm 2010;18:268-274. https://doi.org/10.3109/09273948.2010.490630
10. Stewart MW. The expanding role of vascular endothelial growth factor inhibitors in ophthalmology. Mayo Clin Proc 2012;87:77-88. https://doi.org/10.1016/j.mayocp.2011.10.001

11. Bonanno JA. Molecular mechanisms underlying the corneal endothelial pump. Exp Eye Res 2012;95:2-7. https://doi.org/10.1016/j.exer.2011.06.004

12. Niwa Y, Kakinoki M, Sawada T, Wang X, Ohji M. Ranibizumab and Aflibercept: Intraocular Pharmacokinetics and Their Effects on Aqueous VEGF Level in Vitrectomized and Nonvitrectomized Macaque Eyes. Invest Ophthalmol Vis Sci 2015;56:6501-6505. https://doi.org/10.1167/iovs.15-17279

13. Gharbiya M, Bruscolini A, Sacchetti M, et al. In vivo antivascular endothelial growth factor treatment induces corneal endothelium apoptosis in rabbits through changes in p75NTR-proNGF pathway. J Cell Physiol 2018;233:8874-8883. https://doi.org/10.1002/jcp.26806

14. Akal A, Ulas T, Goncu T, et al. Evaluating the safety of intracameral bevacizumab application using oxidative stress and apoptotic parameters in corneal tissue. Int J Ophthalmol 2015;8:697-702. https://doi.org/10.3980/j.issn.2222-3959.2015.04.10

15. Chiang CC, Chen WL, Lin JM, Tsai YY. Effect of bevacizumab on human corneal endothelial cells: a six-month follow-up study. Am J Ophthalmol 2008;146:688-691. https://doi.org/10.1016/j.ajo.2008.06.002

16. Horozoğlu F, Gönen T, Yaşar M, Sever Ö, Keskinbora K. Comparison of Corneal Endothelial Changes After a Single-Dose Injection of Bevacizumab or Ranibizumab. Turk J Ophthalmol 2013;43:391-394. https://doi.org/10.4274/tjo.82621

17. Park J, Lee M. Short-term effects and safety of an acute increase of intraocular pressure after intravitreal bevacizumab injection on corneal endothelial cells. BMC Ophthalmol 2018;18:17. https://doi.org/10.1186/s12886-018-0682-9

18. Ulutas HG, Yener NP. Effects of Intravitreal Injection on Ocular Surface and Anterior Segment Parameters. Beyoglu Eye J 2021;6:84-89. https://doi.org/10.14744/bej.2021.65487

19. Guzel H, Bakbak B, Koylu MT, Gonul S, Ozturk B, Gedik S. The effect and safety of intravitreal injection of ranibizumab and bevacizumab on the corneal endothelium in the treatment of diabetic macular edema. Cutan Ocul Toxicol 2017;36:5-8. https://doi.org/10.3109/15569527.2016.1140177

20. Lee JS, Oum BS, Choi HY, Lee JE, Cho BM. Differences in corneal thickness and corneal endothelium related to duration in diabetes. Eye (Lond) 2006;20:315-318. https://doi.org/10.1038/sj.eye.6701868

21. Durukan I. Corneal endothelial changes in type 2 diabetes mellitus relative to diabetic retinopathy. Clin Exp Optom 2020;103:474-478. https://doi.org/10.1111/cxo.12971

22. Arslan GD, Guven D, Alkan AA, Kacar H, Demir M. Short term effects of intravitreal anti-vascular endothelial growth factor agents on cornea, anterior chamber, and intraocular pressure. Cutan Ocul Toxicol 2019;38:344-348. https://doi.org/10.1080/15569527.2019.1616749

23. Joshi M, Naik MP, Sarkar L. Effect of intravitreal anti-vascular endothelial growth factor on corneal endothelial cell count and central corneal thickness in Indian population. J Family Med Prim Care 2019;8:2429-2432. https://doi.org/10.4103/jfmpc.jfmpc_314_19

24. Gumus G, Berhuni M, Ozturkmen C. The short-term effects of intravitreal bevacizumab injection on intraocular pressure, cornea, iridocorneal angle, and anterior chamber. Ther Adv Ophthalmol 2022;14:25158414221133772. https://doi.org/10.1177/25158414221133772

25. Güler M, Capkın M, Simşek A, et al. Short-term effects of intravitreal bevacizumab on cornea and anterior chamber. Curr Eye Res 2014;39:989-993. https://doi.org/10.3109/02713683.2014.888452

26. Campa C. Effect of VEGF and anti-VEGF compounds on retinal pigment epithelium permeability: an in vitro study. Eur J Ophthalmol 2013;23:690-696. https://doi.org/10.5301/ejo.5000248

27. Faragher RG, Mulholland B, Tuft SJ, Sandeman S, Khaw PT. Aging and the cornea. Br J Ophthalmol 1997;81:814-817. https://doi.org/10.1136/bjo.81.10.814

28. Raghuram A, Saravanan VR, Narendran V. Intracameral injection of bevacizumab (Avastin) to treat anterior chamber neovascular membrane in a painful blind eye. Indian J Ophthalmol 2007;55:460-462. https://doi.org/10.4103/0301-4738.36484