Original Article

Effect of Intra-Stromal Bevacizumab on Corneal Vascularization

Muhammad Khizar Niazi[1], Hifsa Masood[2], Kashif Ali[3], Saad Mushtaq Malik[4]
Muhammad Asharib Arshad[5]
[1-4]*CMH Multan, CIMS Medical College, NUMS* [5]*The Children's Hospital and institute of child health, Multan*

ABSTRACT

Purpose: To study the effect of Intra-stromal Bevacizumab on corneal vascularization.

Study Design: Quasi experimental study.

Place and Duration of Study: Outpatient department of the Combined Military Hospital, Multan from April 2022 to August 2022.

Methods: Thirty patients were recruited through non-probability, convenience sampling technique. Ethical approval was taken from Institutional Review Board. Complete ocular examination was performed by a consultant ophthalmologist and patients with corneal neovascularization were included in the study. Area of corneal vascularization was measured on slit lamp. After taking all the aseptic measures and administering anesthetic eye drops, patients were administered 0.2 ml of (2.5 mg/0.1 mL) Bevacizumab (Avastin, Roche) using an ophthalmic microscope. The patient was then called for a follow up after 4 weeks and then the grade of corneal vascularization was checked by a consultant ophthalmologist on slit lamp examination. Data analysis was done using SPSS version 25.

Results: Mean age of the patients was 43.4 ± 13.7 years (range 25 to 72 years). Male to female ratio was 2:3. There was significant decrease in the area of corneal neovascularization from 2.78mm ± 0.30 to 2.50mm ± 0.27 (p = 0.0003). The mean change in the area of corneal neovascularization was 10.07%. Two patients had sub-conjunctival hemorrhage which settled over due course of time.

Conclusion: Intra-stromal Bevacizumab resulted in significant reduction in vascularized area of cornea with minimal side effects indicating that *i*ntra-stromal Bevacizumab is an effective and safe modality in the treatment of corneal neovascularization.

Key Words: Cornea, Bevacizumab, VEGF Receptors, Anti-vascular endothelial growth factors.

How to Cite this Article: Niaz MK, Masood H, Ali K, Malik SM, Arshad MA. Effect of Intra-Stromal Bevacizumab on Corneal Vascularization. Pak J Ophthalmol. 2023, **39 (1):** 24-27.

Doi: 10.36351/pjo.v39i1.1550

Correspondence: Muhammad Khizar Niazi
CMH Multan, CIMS Medical College, NUMS
Email: Khizar_aleem@yahoo.com

Received: November 30, 2022
Accepted: December 30, 2022

INTRODUCTION

Cornea is a connective tissue that is avascular and transparent, functioning as a refractive surface and a mechanical barrier of the eye. The blood supply of the cornea is by branches of ciliary arteries. The lucidity of the cornea is due to the complicated balance between the layers and the cellular components of the cornea.[1] Cornea is an immune-privileged structure, which provides protection to the eye and is the reason for successful corneal transplants.[1,2] Corneal neovascularization occurs in various pathologies and can result in compromise of corneal transparency. This results in impaired visual acuity, corneal edema and scarring.[3,4] Cornea being an avascular structure can vascularize due to the imbalance between the anti-angiogenic and angiogenic factors that leads to an excess of proangiogenic factors (such as matrix metalloproteinases, basic fibroblast growth factor

[bFGF] and vascular endothelial growth factor [VEGF]). A deficit in the levels of the anti-angiogenic factors (such as endostatin, angiostatin and VEGF Receptor-2) can lead to the angiogenesis.[5,6] Corneal neovascularization can also occur due to other pathologies which result in corneal hypoxia. Other causes of corneal vascularization include corneal trauma, infections and immunological processes.[7]

VEGF plays an integral role in the pathogenesis of corneal neovascularization.[8,9] Suppression of VEGF levels has been reported as an effective modality for the treatment of corneal neovascularization in literature.[10]

We planned this study to see the effect of Bevacizumab on corneal neovascularization through intra-stromal route.

METHODS

This quasi experimental study was conducted in the outpatient department of Combined Military Hospital, Multan from April 2022 to August 2022. Ethical approval was sought from institutional review board. Sample size was calculated by Raosoft sample size calculator by taking the margin of error as 5%, confidence interval as 95%, population of patients was taken to be 30 and response distribution was 50%. Patients were selected through non-probability, convenience sampling technique. Patients between the age of 25 and 72 years and diagnosed with corneal neovascularization by a consultant ophthalmologist were included in the study. Patients with uncontrolled hypertension, diabetes mellitus, renal disease, liver disease or any coagulation abnormalities were excluded from our study. Other exclusion criteria were history of ocular or periocular malignancy, active corneal ulcer, recent ocular surgery within the last 3 months, active ocular infection at the time of presentation and history of allergy to Bevacizumab.

History and a thorough ocular examination was carried out by a consultant ophthalmologist and the area of corneal neovascularization was measured on slit lamp. Written informed consent was taken from all patients before the procedure. Using aseptic technique and under topical anesthesia, 0.2 ml of (2.5 mg/0.1 mL) of Bevacizumab (Avastin, Roche) was given intra-stromally using a microscope. The patient was called for follow up after 4 weeks and size of corneal neovascularization was checked by the same ophthalmologist. Findings were noted on a proforma. Data was analyzed using SPSS version 25.0 (SPSS for Windows, Chicago, IL). Difference in the size of corneal neovascularization before the administration of Bevacizumab and after four weeks was compared by using a paired t-test and the p-value of less than 0.05 was considered statistically significant.

RESULTS

Thirty eyes of 30 patients (12 males and 18 females) with corneal neovascularization were included in our study. Mean age of the patients was 43.4 ± 13.7 years (range 25 to 72 years). The demographic characteristics of the study population that include the gender, marital status, educational status and occupational status of the patient are listed in Table 1. There was a significant decrease in the area of corneal neovascularization from 2.78 ± 0.30mm before injection and 2.50 ± 0.27mm after Bevacizumab ($p = 0.0003$). Mean change was 10.07%.

DISCUSSION

Vascular endothelial growth factor has an established role in the pathogenesis of corneal neovascularization.[11] In animal models with corneal neovascularization, the levels of expression of vascular endothelial growth factor and its receptors have been found to be increased.[12,13] Research studies conducted on humans have also demonstrated that the expression of vascular endothelial growth factor and its receptors is higher in the cornea of the patients with corneal neovascularization, regardless of the cause.[14] Additionally, the blockage of vascular endothelial growth factor at the level of both mRNA and protein synthesis, has resulted in reduction in corneal neovascularization.[15]

Bevacizumab is a humanized, recombinant, monoclonal immunoglobulin (IgG1) that binds to the Vascular Endothelial Growth Factor A and inhibits its activity, which inhibits the process of angiogenesis.[16] Bevacizumab was the first antibody to be approved by the U.S FDA (Food and Drug Administration) for the treatment of various cancers.[17] Off-label use of Bevacizumab for the treatment of corneal neovascularization gained rapid and widespread acceptance around the world due to its efficacy and safety. In our study, we have demonstrated that Bevacizumab is effective in the treatment of corneal neovascularization.

Vascular endothelial growth factor was formerly considered to be a vascular permeability factor but later on it was discovered that it promotes endothelial growth.[18] It makes microvasculature more permeable to the circulating macromolecules with a much higher potency compared to histamine.[19] Hence, the vascular stabilization provided by anti- VEGF therapy reduces the vascular permeability that results in diminished caliber of the blood vessels. In our study vascular caliber of the corneal neovascularization was also reduced.

There are several theories which explain the variability in response to Bevacizumab on the basis of route and site of administration. Topical method of application of a drug is the preferred method to deliver a drug to the cornea and the ocular surface. However, the topical route of administration of the drug will only be effective if the drug is able to reach the target tissue and the concentration of the drug can reach therapeutic levels. In the case of Bevacizumab, it is a full length immunoglobulin so it is relatively larger to penetrate the intact corneal epithelium. On the contrary, the epithelium covering the area with neovascularization will have increased permeability that results in an incompetent barrier function and helps the drug reach the target tissues and achieve therapeutic concentration.[20] This notion has been supported by recent literature that in animals with corneal neovascularization, Bevacizumab can penetrate cornea and reach the target tissue.[20] Intrastromal administration of Bevacizumab has been shown to be more effective in inducing regression of corneal neovascularization. It has a long duration of action and clearance from the tissue is slow by the normal vasculature of the limbus. This allows the drug to maintain therapeutic concentration for a longer time.[10]

The intrastromal administration of Bevacizumab causes minimal discomfort to the patient and minimal systemic risk has been associated with this route of administration. Gupta et al. reported self-limiting side effects in 3 out of 14 patients.[10] Another report of 6 patients showed no side effects after intrastromal injection of Bevacizumab.[21] In our study only 2 out of 30 patients reported sub-conjunctival hemorrhage. This demonstrates the excellent safety profile of administration of Bevacizumab through the intrastromal route.

Zhang et al. advocated the use of ocular nano systems (ONS) based on nanotechnology for the treatment of corneal neovascularization that has emerged as a great advantage in its treatment during the last two decades.[22] The potential functions of the ocular nanosystems range from nanocarriers, which deliver drugs and genes to target sites in the eye. These ocular nanosystems can be a future perspective for the treatment of corneal neovascularization. However, further research is required in this field so that practical development can be done in the management of corneal neovascularization.

Limitation of this study was small sample size and short duration of follow up. We also did not consider relation of cause of corneal vascularization and response to Bevacizumab. Further research can be conducted along these lines. Different doses of Bevacizumab is another area to be explored.

CONCLUSION

This study provides evidence that intrastromal Bevacizumab is an effective and safe therapeutic modality in the treatment of corneal neovascularization.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval

The study was approved by the Institutional review board/Ethical review board **(ERC No.34/2022).**

REFERENCES

1. **Rates ERD, Almeida CD, Costa EPF, Farias RJM, Santos-Oliveira R, Alencar LMR.** Layer-by-Layer Investigation of Ultrastructures and Biomechanics of Human Cornea. Int J Mol Sci. 2022; **23 (14):**7833. Doi: 10.3390/ijms23147833.
2. **DelMonte DW, Kim T.** Anatomy and physiology of the cornea. J Cataract Refract Surg. 2011; **37 (3):** 588-598. Doi:10.1016/j.jcrs.2010.12.037
3. **Sharif Z, Sharif W.** Corneal neovascularization: updates on pathophysiology, investigations & management. Rom J Ophthalmol. 2019; **63 (1):** 15-22.
4. **Streilein JW.** Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nat Rev Immunol. 2003; **3 (11):** 879-889. doi:10.1038/nri1224
5. **Nicholas MP, Mysore N.** Corneal neovascularization. Exp Eye Res. 2021; **202:** 108363. Doi: 10.1016/j.exer.2020.108363

6. **Chang JH, Gabison EE, Kato T, Azar DT.** Corneal neovascularization. Curr Opin Ophthalmol. 2001; **12 (4):** 242-249. Doi:10.1097/00055735-200108000-00002
7. **Giannaccare G, Pellegrini M, Bovone C, Spena R, Senni C, Scorcia V, et al.** Anti-VEGF Treatment in Corneal Diseases. Curr Drug Targets, 2020; **21 (12):** 1159-1180. Doi: 10.2174/1389450121666200319111710.
8. **Roshandel D, Eslani M, Baradaran-Rafii A, Cheung AY, Kurji K, Jabbehdari S, et al.** Current and emerging therapies for corneal neovascularization. Ocul Surf. 2018; **16 (4):** 398-414. Doi: 10.1016/j.jtos.2018.06.004.
9. **Deli K, Magdalena V, Katerina L, Pavel S.** Treatment of Corneal Neovascularization Using Anti-VEGF Bevacizumab. J Ophthalmol. 2014; **(4):** 178132 Doi: 10.1155/2014/178132
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. Doi: 10.1007/s00417-019-04519-4.
11. **Philipp W, Speicher L, Humpel C.** Expression of vascular endothelial growth factor and its receptors in inflamed and vascularized human corneas. Invest Ophthalmol Vis Sci. 2000; **41 (9):** 2514-2522.
12. **Muellerleile LM, Bernkopf M.** Topical Bevacizumab for the treatment of corneal vascularization in dogs: A case series. Vet Ophthalmol. 2021; **24 (5):** 554-568. Doi: 10.1111/vop.12931.
13. **Ucgul RK, Celebi S, Yilmaz NS, Bukan N, Ucgul AY.** Intrastromal versus subconjunctival anti-VEGF agents for treatment of corneal neovascularization: a rabbit study. Eye (Lond). 2021; **35 (11):** 3123-3130. Doi: 10.1038/s41433-020-01347-3.
14. **Liu S, Romano V, Steger B, Kaye SB, Hamill KJ, Willoughby CE.** Gene-based antiangiogenic applications for corneal neovascularization. Surv Ophthalmol. 2018; **63 (2):** 193-213. Doi: 10.1016/j.survophthal.2017.10.006.
15. **Zhu J, Inomata T, Di Zazzo A, Kitazawa K, Okumura Y, Coassin M, et al.** Role of Immune Cell Diversity and Heterogeneity in Corneal Graft Survival: A Systematic Review and Meta-Analysis. J Clin Med. 2021; **10 (20):** 4667. Doi: 10.3390/jcm10204667
16. **Ferrara N.** Commentary on Humanization of an Anti-VEGF Monoclonal Antibody for the Therapy of Solid Tumors and Other Disorders. Cancer Res. 2016; **76 (17):** 4913-4915.doi: 10.1158/0008-5472.CAN-16-1973.
17. **Keating AM, Jacobs DS.** Anti-VEGF Treatment of Corneal Neovascularization. Ocul Surf. 2011; **9 (4):** 227-237. Doi: 10.1016/s1542-0124(11)70035-0.
18. **Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, et al.** Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest. 1989; **84 (5):** 1470-1478. Doi: 10.1172/JCI114322.
19. **Wautier JL, Wautier MP.** Vascular Permeability in Diseases. Int J Mol Sci. 2022; **23 (7):** 3645. Doi:10.3390/ijms23073645
20. **Yoeruek E, Ziemssen F, Henke-Fahle S, Tatar O, Tura A, Grisanti S, et al.** Safety, penetration and efficacy of topically applied Bevacizumab: evaluation of eye drops in corneal neovascularization after chemical burn. Acta Ophthalmol. 2008; **86 (3):** 322-328. Doi: 10.1111/j.1600-0420.2007.01049.x
21. **Vieira AC, Höfling-Lima AL, Gomes JÁ, Freitas Dd, Farah ME, Belfort R Jr.** Intrastromal injection of Bevacizumab in patients with corneal neovascularization. Arq Bras Oftalmol. 2012; **75 (4):** 277-279. Doi:10.1590/s0004-27492012000400012
22. **Zhang C, Yin Y, Zhao J, Li Y, Wang Y, Zhang Z, et al.** An Update on Novel Ocular Nanosystems with Possible Benefits in the Treatment of Corneal Neovascularization. Int J Nanomedicine, 2022.; **17:** 4911-4931. Doi: 10.2147/IJN.S375570.

Authors' Designation and Contribution

Muhammad Khizar Niazi; Professor: *Concepts, Design, Literature search, Data acquisition, Manuscript preparation, Manuscript editing, Manuscript review.*

Hifsa Masood; *Concepts, Design, Literature search, Statistical analysis, Manuscript preparation.*

Kashif Ali; Assistant Professor: *Data acquisition, Data analysis, Statistical analysis, Manuscript review.*

Saad Mushtaq Malik; Assistant Professor: *Data acquisition, Data analysis, Manuscript editing, Manuscript review.*

Muhammad Asharib Arshad; Medical Officer: *Data analysis, Statistical analysis, Manuscript editing, Manuscript review.*