Original Article

Effect of intravitreal anti-vascular endothelial growth factor on corneal endothelial cell count and central corneal thickness in Indian population

Mukesh Joshi[1], Mayuresh P. Naik[2], L. Sarkar[1]

[1]Department of Ophthalmology, V.M.M.C and Safdarjung Hospital, Ring Road, Ansari Nagar, [2]Department of Ophthalmology, H.I.M.S.R and H.A.H. Centenary Hopsital, Nears GK-2, Alaknanda, New Delhi, India

Abstract

Purpose: To evaluate the effect of intravitreal anti-VEGF on corneal endothelial cell count and central corneal thickness as well as to compare these in phakic and pseudophakic eyes. **Material and Methods:** The study was conducted in 102 eyes selected, as per selection criteria, over a time period of 18 months. At first patient visit, examination included: 1. Fundus examination. 2. Specular microscopy was done to look for endothelial cell count and Central corneal thickness. At second visit, Injection 0.5 mg/0.05 ml of ranibizumab was administered. Visits at day 1, day 7 and 1 month were done for Endothelial cell density and central corneal thickness was measured by specular microscope. **Results:** The mean CCT value in pseudophakic group was 502.08 ± 19.91, 501.9 ± 20.31, and 501.72 ± 21.55 on day 1, 7 and 30, respectively. The mean CCT value in phakic group was 506.53 ± 22.61, 505.96 ± 20.12, 505.92 ± 20.3 and 505.69 ± 21.47. The mean value of ECD in pseudophakic eyes on day 1, 7, and 30 were 2284.24 ± 299.86, 2281.39 ± 289.46 and 2284.06 ± 312.65 cells/mm^2, respectively. The mean value of ECD in phakic eyes on day 1, 7, and 30 were 2314.51 ± 212.08, 2313.92 ± 212.7 and 2313.63 ± 216.86 cells/mm^2, respectively. **Conclusion:** There is no significant change in endothelial cell density, central corneal thickness, coefficient of variation and intraocular pressure before and after intravitreal injection over one month of follow-up. The results are similar between phakic and pseudophakic eyes.

Keywords: Anti-VEGF, central corneal thickness, endothelial cell count

Introduction

Vascular Endothelial Growth Factor has been identified as an endothelial cell-specific mitogen and angiogenic stimulator *in vivo*. Multiple biologically active forms of VEGF-A are generated by both alternative mRNA splicing and posttranslational modification (proteolytic cleavage), and two of these forms (VEGF165 and VEGF121) have been detected in choroidal neovascular lesions. Anti-VEGFs (namely Ranibizumab, Bevacizumab, Pegabtinib) are stable small RNA-like molecules that bind to the 165-kDa isoform of human VEGF and inactivate it, thereby reducing the retinal and choroidal angiogenesis and halting the increase in vascular permeability.[1]

Intracameral injections of bevacizumab for treatment of neovascular glaucoma can cause corneal endothelial cell loss as determined by specular microscopy, although the endothelial cell loss is less as compared to that occurring after cataract surgeries or glaucoma surgeries.[2] Although the pharmacokinetic profile of ranibizumab after intravitreal injection in human eyes has not been determined definitively, ranibizumab has been detected in the aqueous humor after intravitreal standard injections in animal models.[1,2] Moreover, VEGF and its receptors were expressed in the corneal endothelium.[3-6] Therefore, ranibizumab

Address for correspondence: Dr. Mayuresh P. Naik, Department of Ophthalmology, H.I.M.S.R and H.A.H. Centenary Hopsital, Room No. 3 of Eye OPD, 1st Floor of OPD Building, Near GK-2, Alaknanda, New Delhi - 110 062, India.
E-mail: mayureshpnaik@gmail.com

Received: 18-04-2019 **Revised:** 19-04-2019 **Accepted:** 06-05-2019

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_314_19

How to cite this article: 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;8:2429-32.

in the aqueous humor after intravitreal injections may affect the function of VEGF in the corneal endothelium.

There has been a paucity of studies to elucidate the possible endothelial toxicity of anti-VEGF molecules in the Indian population, and it is this void that we have attempted to fill in this study.

Materials and Methods

The study was conducted prospectively over a time period of 18 months. 102 eyes requiring intra-vitreal anti-VEGF drugs for macular oedema, age-related macular degeneration or retinal vein occlusion were selected (as per selection criteria) for evaluation and a comparative follow-up. Sample size was calculated in order to achieve a power of study 80% at $P < 0.05$ based on the number of patents attending the speciality retina clinic of our tertiary health care centre (i.e. keeping in mind the prevalence of the etiologies in question). Institutional Review Board (IRB)/ Ethics Committee approval was obtained.

Patients aged 40-80 years who were willing to come for regular follow-up, were included in the study if they demonstrated focal or diffuse macular edema on Optical Coherence Tomography (OCT) due to age-related macular degeneration or retinal venous occlusion requiring intravitreal anti-VEGF injection.

Patients were excluded if they had pre-existing glaucoma or glaucoma shunt surgery, aphakic and vitrectomised patients, corneal scarring or preexisting corneal disease like Fuchs endothelial dystrophy, that could lower endothelial cell count, contact lens users, any ocular trauma that disrupts the structural integrity of cornea, previous recipients of anti-VEGF injection and uncooperative patients who were unable to perform specular microscopy.

At first patient visit, a detailed medical history was obtained. Furthermore, the following examinations were performed:

1. Snellen's best corrected visual acuity (BCVA)
2. Intraocular pressure (IOP) was measured with non contact tonometry (NCT)
3. Slit lamp examination of anterior segment.
3. Fundus examination
4. Specular microscopy (Konan Noncon Robo NSP-7700) was done to look for endothelial cell count and Central corneal thickness. An average of 3 readings were taken. Patients were asked always to look at the central fixation target and the auto-alignment function was used. All corneal endothelial cells which were clearly visible on the picture were marked manually. Endothelial cell density (ECD) were calculated by the instruments' built-in software after marking 20 discrete points.

At second visit, injection ranibizumab 0.5 mg/0.05 ml was administered through the pars plana into the vitreous cavity using a 30G needle at a limbal distance of 3.5 mm in pseudophakics and 4 mm in phakics.

Post-operatively, the patient was given Tablet acetazolamide 250 mg 2 tablets stat, E/D moxifloxacin 0.5% QID × 7 days and E/D bromfenac 0.09% BD × 7 days.

3 more visits were performed at 1 day, 7 days and 1 month post-operatively, to quantify endothelial cell density and central corneal thickness by specular microscopy.

Statistical analysis

Patients were divided into 2 groups for the purpose of evaluation:
Group 1: Phakic patients.
Group 2: Pseudophakic patients.

Categorical variables were presented in number and percentage (%) and continuous variables was presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then non-parametric test was used with the power of the study being 80% such that $P < 0.05$ being considered statistically significant.

The statistical tests were applied as follows:

1. Quantitative variables were compared using Wilcoxon ranked sum test to compare pre with post test results and independent T test/Mann whitney Test (for non parametric data) to compare two groups.
2. Qualitative variables were compared using Chi-Square test/ Fisher's exact test.

A P value of < 0.05 was considered statistically significant.

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Results

Patients were divided into 2 groups for the purpose of evaluation:
Group 1: Phakic patients.
Group 2: Pseudophakic patients.

Since both groups were age and sex matched, there was not much difference between them with respect to the indications for intravitreal injections. Ref. Table 1.

2 patients were lost to follow up due to death, and so the final statistical analysis was carried out on 100 patients.

There was no significant difference between the two groups in terms of mean age ($P = 0.269$), pre injection central corneal thickness (CCT) ($P = 0.632$), pre injection endothelial cell density (ECD) ($P = 0.864$), pre injection coefficient of variation (CV) ($P = 0.350$). Ref. Table 1.

The mean CCT value in phakic group was 506.53 ± 22.61, 505.96 ± 20.12, 505.92 ± 20.3 and 505.69 ± 21.47. The mean CCT values in phakics decreased by 0.07% ± 2.08%, 0.07% ± 2.60% and

0.12% ± 2.60% day 1, 7 and 30, respectively. This change in mean CCT value was not statistically significant ($P > 0.05$). Ref. Table 2.

The mean CCT value in pseudophakic group was 502.08 ± 19.91, 501.9 ± 20.31, and 501.72 ± 21.55 on day 1, 7 and 30, respectively. The mean CCT values decreased by 0.34% ± 3.03%, 0.36% ± 3.46% and 0.40% ± 3.65% on day 1, 7 and 30, respectively. This change in mean CCT was not statistically significant ($P > 0.05$). Ref. Table 2.

There was also no significant difference between the phakic and pseudophakic patients in terms of change in mean CCT values ($P > 0.05$). Ref. Table 2.

The mean value of ECD in phakic eyes on day 1, 7, and 30 were 2314.51 ± 212.08, 2313.92 ± 212.7 and 2313.63 ± 216.86 cells/mm^2, respectively. It reduced by 0.02% ± 1.39%, 0.00% ± 1.54% and 0.04% ± 1.41%, respectively. The decrease however was not statistically significant ($P > 0.05$). Ref. Table 3.

The mean value of ECD in pseudophakic eyes on day 1, 7, and 30 were 2284.24 ± 299.86, 2281.39 ± 289.46 and 2284.06 ± 312.65 cells/mm^2, respectively. It decreased by 0.06% ± 2.98%, 0.08% ± 3.66% and 0.16% ± 3.79%, respectively, on day 1, 7 and 30, respectively. The decrease however was not statistically significant ($P > 0.05$). Ref. Table 3.

There was no significant difference between phakic and pseudophakic eyes in terms of ECD ($P > 0.05$). Ref. Table 3.

Discussion

Although the pharmacokinetic profile of ranibizumab after intravitreal injection in human eyes has not been determined definitively, ranibizumab has been detected in the aqueous humor after intravitreal standard injections in animal models.[1,2] In a rabbit study, ranibizumab was present in the aqueous humor of eyes after a 0.5-mg intravitreal injection of ranibizumab but was not detected in the serum or the fellow untreated eyes. Concentrations exceeding 0.1 mg/mL of ranibizumab were maintained in the ocular compartments for 29 days.

Previously, many studies have shown that VEGF and its receptors are expressed in cornea. Gan *et al.*[7] in 2004 found out that VEGF is expressed in rabbit eyes in corneal epithelium, and endothelium but not in corneal stroma. They also observed that VEGFR-2 was present in the corneal epithelial and endothelial cells but absent in keratinocytes. Phillip *et al.*[8] also found out that VEGF and VEGFR are present in corneal epithelium and endothelium but not on keratinocytes, and that their expression was increased in patients with inflamed and injured cornea, thereby indicating that VEGF may be involved in neovascularisation of injured and inflamed human corneas.

Yoeruek *et al.*[9] in 2007 did an *in vitro* study and reported that all human corneal cell lines were immunopositive for VEGF, VEGFR-1 and VEGFR-2. It is therefore reasonable to consider that ranibizumab may have cytotoxic effects on corneal endothelium.

J. Benitz herreros *et al.*[10] in 2010 conducted the morphometric analysis of corneal endothelium after 0.5 mg intravitreal ranibizumab injection in AMD patients using corneal specular microscopy on American population. In their study, there was no significant difference in the corneal endothelial cell density and central corneal thickness at 6 months. Consuelo Perez-Rico *et al.*[11] conducted a prospective observational case series study in 2010 on effect of intravitreal ranibizumab on corneal endothelium in patients of age-related macular degeneration, which showed that there was no significant effect on endothelial cell count, coefficient of variation, % of hexagonal cells and central corneal thickness after a follow-up of 6 months. Chun-Chi Chiang *et al.*[12] in 2008 and Tseng JJ *et al.*[13] in 2012 conducted a six monthly follow-up study to determine the effect of 2.5 mg intravitreal bevacizumab on central corneal thickness. This study showed that there was no significant difference in central corneal thickness before and after injection. Our study results indicated that intravitreal injection of 0.5 mg of anti-VEGF in patients with AMD or macular oedema secondary to RVO did not cause a significant change in central corneal thickness, endothelial cell density, coefficient of variation as compared to the preinjection values, as measured by specular

Table 1: Baseline parameters

VARIABLES	GROUP 1 PSEUDOPHAKIC	GROUP 2 PHAKIC	*P*
MEAN AGE	62.35±6.55	60.86±6.99	0.269
PRE INJECTION CCT	503.9±19.1	506.5±22.6	0.632
PRE INJECTION ECD	2287.1±303.4	2314.7±218.1	0.864
INDICATION FOR INTRAVITREAL INJECTION			
1. ARMD	29	31	
2. VASCULAR OCCLUSIONS	21	19	

Table 2: Comparision of pre injection and post injection (day 1, 7 & 30) CCT (um) between pseudophakics and phakic group

PARAMETER	GROUP 2 PSEUDOPHAKIC	GROUP 1 PHAKIC	*P* (intergroup)
PREINJECTION CCT	503.9±19.1	506.5±22.6 (−0.07%±2.08%)	0.632
CCT day 1	502.0±19.9 (−0.34%±3.03%)	505.9±20.1 (−0.07%±2.60%)	0.328
CCT day 7	501.9±20.3 (−0.36%±3.46%)	505.9±20.3	0.298
CCT day 30	501.7±21.5 (−0.40%±3.65%)	505.6±21.4 (−0.12%±2.60%)	0.416

Table 3: Comparison of pre injection and post injection (day 1, 7 & 30) ECD (cells/mm^3) between pseudophakics and phakic group

PARAMETER	GROUP 1 PSEUDOPHAKIC	GROUP 2 PHAKIC	*P*
Pre injection ECD	2287.1±303.4	2314.7±218.1	0.864
ECD day 1	2284.2±299.8 (−0.06%±2.98%)	2314.5±212.0 (−0.02%±1.39%)	0.899
ECD day 7	2281.3±289.4 (−0.08%±3.66%)	2313.9±212.7 (−0.00%±1.54%)	0.925
ECD day 30	2284.0±312.6 (−0.16%±3.79%)	2313.6±216.8 (−0.04%±1.41%)	0.606

microscopy over 1 month of follow-up, which is in accordance with the above review of previous studies.

We would now like to talk about the strengths of the study. Our study strongly supports that a single intravitreal injection of anti-VEGF (0.5 mg ranibizumab) does not seem to cause significant changes in corneal endothelium and intraocular pressure. Also, unlike many other similar studies with smaller sample sizes, the statistical significance and validity of this study is corroborated by the fact that we had a sample size of over 100 patients.

However, there are a few weaknesses to the study. Firstly, most patients end up requiring more than one intravitreal injection to attain anatomical and functional integrity of the retina. Hence, this study applies to that section of patients requiring a single dose intravitreal anti-VEGF injection and the effect of multiple doses of intravitreal anti-VEGF injections need to be investigated. Secondly, even though we could conclude that a single intravitreal injection may not harm the cornea adversely, it cannot be extrapolated to patients with mildly or partially decompensated corneas wherein even a single injection might affect the endothelium adversely. Thirdly, the results of this study are confined to one month of follow-up. The long term effects of intravitreal anti-VEGF may still require further evaluation. And lastly, this study cannot be extrapolated to aphakic vitrectomised patients wherein the intravitreal anti-VEGF is amenable to direct access to the anterior chamber.

General practitioners and family physicians are generally the first point of contact for healthcare especially in the Indian set-up. Besides careful history taking and examination, the diagnosis and follow-up of patients of wet ARMD demands a very high standard of patient compliance. Since the patient may not be able to experience the beneficial results of an intravitreal immediately and also because the patient might probably require more than a single intravitreal injection, the responsibility of proper counselling and compliance generally falls on the shoulders of these general practitioners and family physicians.

Conclusion

No significant change in endothelial cell density and central corneal thickness was found after intravitreal injection over one month of follow-up, neither in phakic nor in pseudophakic eyes.

Thus, intravitreal anti-VEGF injection appears to be safe on cornea in phakic and pseudophakic eyes in terms of corneal endothelial cell count and central corneal thickness.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Gaudreault J, Fei D, Rusit J, Suboc P, Shiu V. Preclinical pharmacokinetics of ranibizumab (rhuFabV2) after a single intravitreal administration. Invest Ophthalmol Vis Sci 2005;46:726-33.
2. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Ezzat MK, Singh RJ. Pharmacokinetics of intravitreal ranibizumab (Lucentis). Ophthalmology 2007;114:2179-82.
3. Chang JH, Garg NK, Lunde E, Han KY, Jain S, Azar DT. Corneal neovascularization: An anti-VEGF therapy review. Surv Ophthalmol 2012;57:415-29.
4. Pan Z, Fukuoka S, Karagianni N, Guaiquil VH, Rosenblatt MI. Effect of VEGF on corneal nerve growth and corneal wound healing. Invest Ophthalmol Vis Sci 2012;53:3561.
5. Yoeruek E, Spitzer MS, Tatar O, Aisenbrey S, Bartz-Schmidt KU, Szurman P. Safety profile of bevacizumab on cultured human corneal cells. Cornea 2007;26:977-82.
6. Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, *et al.* Corneal avascularity is due to soluble VEGF receptor-1. Nature 2006;443:993-7.
7. 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;82:557-63.
8. 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;41:2514-22.
9. Yoeruek E, Spitzer MS, Tatar O, Aisenbrey S, Bartz-Schmidt KU, Szurman P. Safety profile of bevacizumab on cultured human corneal cells. Cornea 2007;26:977-82.
10. Benitz-Herreros J, Perez-Rico C, Teus MA, Gomez-San Gil Y, Castro-Rebollo M. Morphometric analysis of corneal endothelium after intravitreal ranibizumab in age related macular degeneration treatment. Arch Soc Esp Optalmol 2010;85:329-32.
11. Pérez-Rico C, Benitez-Herreros J, Castro-Rebollo M, Gomez-Sangil Y, Germain F, Montes-Mollon MA, *et al.* Endothelial cell analysis after intravitreal rannibizumab in age related macular degeneration treatment: A pilot study. Br J Ophthalmol 2010;94:267-8.
12. Chiang CC, Chen WL, Lin JM, Tsai Y. Effect of bevacijumab on human corneal endothelial cells-A six monthly follow up study. Am J Ophthalmol 2008;146:688-91.
13. Tseng JJ, Vance SK, Torre KE, Mendonca LS, Cooney MJ, Klancnik JM, *et al.* Sustained IOP elevation due to anti-VEGF therapy. J Glaucoma 2012;21:241-7.