Comparison of Ranibizumab and Aflibercept Effects on Inner Retinal Layers in Neovascular Age-Related Macular Degeneration

Nur Demir[1], Belma Kayhan[2], Sukru Sevincli[2], Murat Sonmez[2]

ABSTRACT

Purpose: To evaluate the changes of all inner retinal layers in eyes with neovascular age-related macular degeneration (nAMD) during anti-vascular endothelial growth factor (anti-VEGF) treatments and to compare two different anti-VEGF agents; ranibizumab and aflibercept.

Materials and Methods: This retrospective, comparative study comprised 40 treatment-naive eyes of 40 patients treated with ranibizumab (20 eyes) or aflibercept (20 eyes) injections for nAMD. Thicknesses of the total retina and inner retinal layers were measured by spectral-domain OCT in the central 1-mm diameter and surrounding 3-mm diameter rings of Early Treatment Diabetic Retinopathy Study grid. Changes in each layer and comparison between ranibizumab and aflibercept groups were analyzed statistically during 1-year follow-up.

Results: The ranibizumab group showed 3.85±16.54 letters of increase and the aflibercept group showed 4.20±16.30 letters of increase at the end of 1 year. There was a significant decrease in total retinal thickness in both groups at the end of 1 year (p=0.006 in ranibizumab, p=0.005 in aflibercept). Ganglion cell layer thinning was significant at the end of 3 months, thinning of the inner nuclear layer was significant at the end of one year in both groups (p<0.05). There was no statistically significant difference between ranibizumab and aflibercept groups.

Conclusions: The thicknesses of all inner retinal layers diminish one year after anti-VEGF treatment of nAMD in both ranibizumab and aflibercept groups due to therapeutic effect obtained with fluid resorption, but not related to tissue loss. However, there are no statistically significant differences in changes of inner retinal layer thicknesses between two anti-VEGF agents.

Keywords: Anti-vegf, ranibizumab, aflibercept, inner retinal layers, ganglion cell layer.

INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of blindness among people older than 50 years in developed countries.[1,2] Its prevalence increases as a result of prolongation in the lifespan and aging of the population. A study reported that 8.7% of the worldwide population had age-related macular degeneration, and the projected number of people with the disease was around 196 million in 2020, increasing to 288 million in 2040.[3]

Vascular Endothelial Growth Factor (VEGF) is one of the most important proangiogenic factors in the body, which also increases microvascular permeability. VEGF plays a major role in abnormal angiogenesis in AMD. VEGF blockage by anti-VEGF agents inhibits angiogenesis and vascular leakage .[4,5] Therefore, anti-VEGF agent injections are widely used treatments in neovascular AMD (nAMD) currently. Several studies state the very good efficacy of anti-VEGF treatment in visual improvement in nAMD patients but this treatment requires many injections to maintain structural and visual effects.[6,7]

VEGF has an important contribution to normal physiological processes to maintain healthy tissue. Moreover, endogenous VEGF has an important role in the maintenance and function of adult retinal neuronal cells.[8,9] Increasing the duration of treatment and the amount of the injected anti-VEGF agent can probably induce some adverse effects on normal tissues.[10] Studies report the cytotoxic effect of anti-VEGF agents on neurons and glial cells.[11] In a study done in a diabetic rat model, inhibition

1- MD, The University of Health Sciences, Sultan 2. Abdulhamid Han Training and Research Hospital, Department of Ophthalmology, Istanbul, Turkey

2- Professor, MD, The University of Health Sciences, Sultan 2. Abdulhamid Han Training and Research Hospital, Department of Ophthalmology, Istanbul, Turkey

Received: 04.06.2021
Accepted: 21.08.2021
Ret-Vit 2021; 31: 111-117
DOİ:10.37845/ret.vit.2022.31.19

Correspondence Adress:
Nur Demir
The University of Health Sciences, Sultan 2. Abdulhamid Han Training and Research Hospital, Department of Ophthalmology, Istanbul, Turkey
Phone: +90 530 5912355
E-mail: nurd9920@gmail.com

of VEGF significantly increased retinal ganglion cell apoptosis and neuronal cell apoptosis in the inner nuclear layer and led to neuronal cell death in the inner retina.[12]

Optical coherence tomography (OCT) is a standard imaging tool in retinal disorders. AMD is also a disease where OCT is used invariably to define the disease and to determine the treatment. Although the latest software enables to the analysis of the retinal layers by automatic segmentation, they may be inaccurate in discrimination of every retinal layer in eyes with AMD where a normal structure is disrupted. Therefore, manual correction of the retinal layer lines allows accurate segmentation and analyses of retinal layers.[13] In this study, we aimed to evaluate the effects of two different anti-VEGF treatments; ranibizumab and aflibercept on inner retinal layers of eyes with nAMD by using Spectral-Domain OCT (SD-OCT) and by correcting errors of automatic segmentation.

MATERIALS AND METHODS

This retrospective, comparative study comprised 40 treatment-naive eyes of 40 patients. The records of patients who were treated for nAMD in the Ophthalmology Department between 2015- 2018 were analyzed. The study was approved by the Ethical Committee of the hospital (Approval number: 46418926-050.03.04) and performed in adherence with the tenets of the Declaration of Helsinki. Written informed consent was taken from all participants.

Patients who were treatment-naive with the diagnosis of nAMD at presentation and who received ranibizumab (Lucentis; Novartis Pharma, Basel, Switzerland) or aflibercept (Eylea; Regeneron, Tarrytown, NY, USA) treatments in only one eye were included in the study. Exclusion criteria were the history of any intervention other than cataract surgery, such as laser photocoagulation, photodynamic therapy, or any previous intravitreal injection; the diagnosis of glaucoma, any ocular inflammation, or any other retinal diseases such as diabetic retinopathy, retinal vascular diseases; refractive errors more than 6 diopters; age younger than 50 years. Eyes with polypoidal choroidal vasculopathy and retinal angiomatous proliferation were identified and excluded by fundoscopy, OCT, fundus fluorescein angiography (FFA), and/or indocyanine green angiography. Uncontrolled systemic hypertension and diabetes mellitus were the other reasons for exclusion. The records with a follow-up duration of less than 12 months were also excluded.

Files of 308 patients with nAMD diagnosis were reviewed. Files of 72 patients were excluded because they had a history of previous anti-VEGF injections in other clinics. In the second step, 85 eyes were eliminated according to the exclusion criteria that we reported in the article previously. Additionally, patients who do not meet follow-up and single-type injection criteria were excluded from the study (74 eyes). 37 eyes where OCT scanning technique had not been raster or scans hadn't clear image were also eliminated finally. Forty nAMD treatment-naive patients who met all eligibility criteria were included in the study and evaluated for statistical analysis, as 20 eyes in the ranibizumab group and 20 eyes in the aflibercept group. We manually corrected inner layers and basement membrane in nearly all scans (25 X 3 for every patient in the study group).

Patients received three monthly intravitreal injections of 0.5 mg ranibizumab or 2 mg aflibercept and were treated according to the Pro Re Nata regimen. To detect and treat any possible complication after each injection, control visits were initiated on the first post-injection day and maintained until any complication, especially ocular tension rise, was eliminated. In case that intraocular pressure (IOP) raised over 25 mmHg, anti-glaucomatous medication was administered. Records of complete ophthalmological examination, IOP, and SD-OCT evaluation performed before treatment, 1 month after 3rd injection, and at the end of one year were analyzed. Visual acuity was tested with an Early Treatment Diabetic Retinopathy Study (ETDRS) chart.

Retinal OCT was performed by use of Spectralis SD-OCT (Version 1.10.4.0, Software_V6.16.2, Heidelberg Engineering, Heidelberg, Germany). Images were acquired by an experienced operator. Well-centered images with good quality signals were selected for analysis. An SD-OCT macular examination was performed without pupil dilation. The scan was conducted on 30×20 degrees of a cube with 25 raster lines separated by 240 µ. Firstly, the retinal layer segmentation was made automatically, and then, the erroneous lines were manually corrected and these calculations were recorded (Figure1). Total retinal thickness, thicknesses of the macular retinal nerve fiber layer (mRNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), and inner nuclear layer (INL) were measured in the central 1-mm diameter subfield (R1) and surrounding 3-mm diameter region (R3) with superior, inferior, temporal and nasal subfields of ETDRS grid. Total macular thickness and the thicknesses of each retinal layer in the inner 3-mm diameter region were calculated as the mean of the thicknesses in four subfields.

SPSS 20.0 (Statistical Package for Social Sciences lnc, Chicago, IL) was used for statistical analysis and interpretation of the data. Continuous variables of descriptive statistical methods were reported as the mean and standard deviation. Categorical variables of descriptive variables were reported as a percentage. The compatibility of the quantitative data to normal distribution

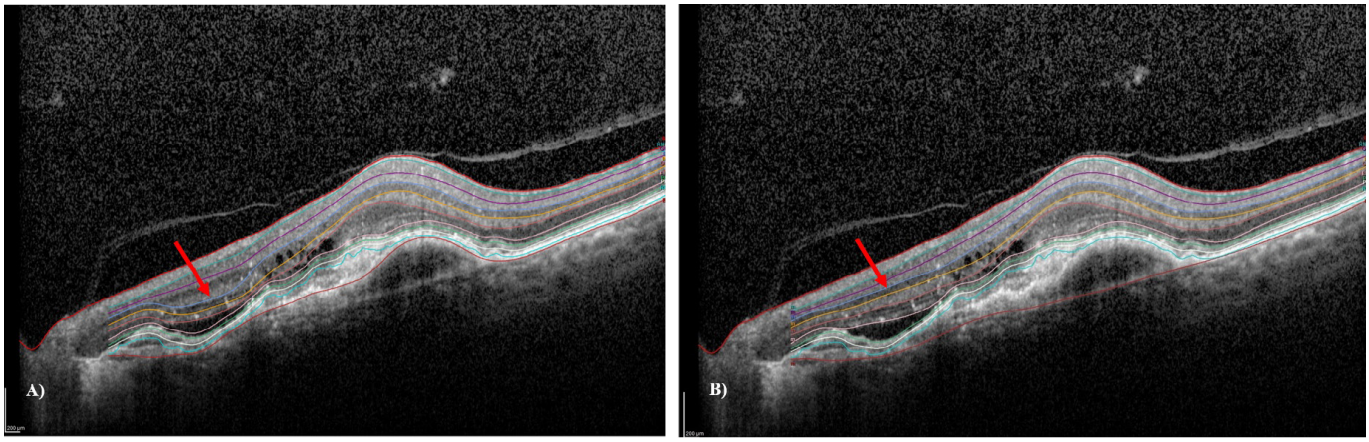

Figure 1: A) *Automated segmentation of macular retinal layers on OCT in a case with nAMD. The red arrow shows the improper delineation of retinal layers.* **B)** *Manual correction of retinal layer segmentation errors. The red arrow shows the manually corrected delineation in the same case.*

was analyzed with the Shapiro-Wilk test. Paired t-test for normally distributed variables was applied for analyses differences for the comparison of the results across the pre- and the post-. P values of less than 0.05 were considered statistically significant.

RESULTS

Our study comprised 40 eyes of 40 patients; 20 eyes in the ranibizumab group and 20 eyes in the aflibercept group. There were no statistically significant differences between groups, related to patient demographics and baseline characteristics (Table 1).

Best-corrected visual acuity (BCVA) in the ranibizumab group demonstrated an increase of 5.00±11.36 letters and 3.85±16.54 letters at the end of 3rd month and of 1 year respectively. BCVA in the aflibercept group increased 7.40±13.55 letters and 4.20±16.30 letters at the end of the 3rd month and one year respectively. There was no statistically significant difference between groups (p› 0.05). IOP measurements didn't show any statistically significant change within each group and between ranibizumab and aflibercept groups during follow-up [13.15±1.53 mmHg at baseline, 13.05±1.70 mmHg after 1 year (p=0.823) in ranibizumab group; 12.90±2.07 mmHg at baseline, 13.10±1.86 mmHg after 1 year (p=0.725) in aflibercept group; p=0.930 between groups after 1 year].

Baseline values of total retinal thickness and thicknesses of inner retinal layers were comparable between the two groups (p› 0.05) (Table 2).

Thickness changes of the total retina and each inner retinal layer were evaluated in the R3 area. Total retinal thickness difference was statistically significant in each

Table 1: *Patient demographics and baseline characteristics.*

	Ranibizumab	**Aflibercept**	**p**
Gender (n, %)	9 (45%) Female	10 (50%) Female	0.752*
	11 (55%) Male	10 (50%) Male	
Age (year) (mean ± SD)	74.85 ± 12.61	73.95 ± 10.05	0.804**
Baseline BCVA (ETDRS letters/logMAR) (mean ± SD)	56.65 ± 15.50/ 0.56 ± 0.31	55.35 ± 15.39/ 0.59 ± 0.30	0.792**
Pseudophakia (n, %)	9 (45%)	8 (40%)	0.749*
Number of Intravitreal Injections (mean ± SD)	5.85 ± 1.72	5.08 ± 1.64	0.156**
Intraocular Pressure (mmHg) (mean ± SD)	13.15 ± 1.53	12.90 ± 2.07	0.667**
SRF (n)	5	6	0.723*
IRF (n)	8	4	0.168*
SRF + IRF	7	10	0.337*

*Pearson Chi Square test, **Independent Samples t-test
BCVA, best corrected visual acuity; SRF, subretinal fluid; IRF, intraretinal fluid.

Table 2: *Baseline thickness values of total retina and inner retinal layers.*

	Ranibizumab n=20	Aflibercept n=20	p*
TRT (R1), µ	398.95 ± 83.78	406.40 ± 89.71	0.788
TRT (R3), µ	390.25 ± 57.33	396.98 ± 56.30	0.710
RNFL (R1), µ	15.60 ± 4.16	15.75 ± 3.62	0.904
RNFL (R3), µ	22.78 ± 3.31	25.12 ± 4.65	0.075
GCL (R1), µ	19.05 ± 5.55	20.75 ± 7.51	0.421
GCL (R3), µ	40.70 ± 6.46	44.19 ± 5.55	0.075
IPL (R1), µ	25.65 ± 7.91	26.30 ± 8.14	0.799
IPL (R3), µ	37.43 ± 6.30	41.04 ± 6.25	0.077
INL (R1), µ	30.70 ± 8.50	33.25 ± 10.52	0.405
INL (R3), µ	41.79 ± 5.18	44.49 ± 8.24	0.223

*Independent samples t-test.
Values are presented as mean ± standard deviation.
TRT, total retinal thickness; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; R1, central 1 mm diameter area of ETDRS grid; R3, inner 3 mm diameter ring area of ETDRS grid.

group at the end of 3rd month and one year. Although all layers demonstrated thinning during follow-up period, the difference was not statistically significant in each control point (Table 3). RNFL thinning was significant in only the ranibizumab group at the end of one year. GCL thinning was significant in both groups at the end of 3rd month.

Table 3: *Changes of retinal layer thicknesses at the end of 3rd month and 1 year in R3 area in each group.*

	Ranibizumab n=20	p*	Aflibercept n=20	p*
TRT 0-3 m	-32.02 ± 64.36	***0.038***	-50.35 ± 86.94	***0.018***
TRT 0-1 y	-44.65 ± 64.90	***0.006***	-50.95 ± 71.30	***0.005***
RNFL 0-3 m	-0.54 ± 4.01	0.554	-2.19 ± 5.56	0.094
RNFL 0-1 y	-1.12 ± 2.12	***0.029***	-2.00 ± 5.01	0.091
GCL 0-3 m	-1.24 ± 2.51	***0.040***	-2.03 ± 3.78	***0.027***
GCL 0-1 y	-2.10 ± 2.59	***0.002***	-2.40 ± 5.39	0.061
IPL 0-3 m	-0.19 ± 5.01	0.867	-3.37 ± 5.34	***0.011***
IPL 0-1 y	-1.14 ± 3.88	0.205	-2.48 ± 5.30	0.050
INL 0-3 m	-2.54 ± 8.96	0.220	-5.78 ± 8.71	***0.008***
INL 0-1 y	-3.62 ± 6.86	***0.029***	-6.25 ± 9.68	***0.009***

*Dependent samples t-test.
Values are presented as mean ± standard deviation and in microns.
TRT, total retinal thickness; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; 0-3 m, between baseline and the end of 3rd month; 0-1 y; between baseline and the end of 1 year.

However, the thickness change in INL was significant in two groups at the end of one year (Table 3).

The mean change of total retinal layer and inner retinal layer thicknesses from baseline to the end of 3rd month and of one year didn't show a statistically significant difference between groups in the R1 area (Table 4).

The mean change of total retinal layer and inner retinal

Table 4: *Comparison of retinal layer thickness changes in R1 area at the end of 3rd month and 1 year.*

	Ranibizumab n=20	Aflibercept n=20	p*
TRT 0-3 m	-39.65 ± 102.49	-60.00 ± 118.80	0.565
TRT 0-1 y	-64.10 ± 103.12	-70.00 ± 109.58	0.862
RNFL 0-3 m	-0.60 ± 6.26	-1.45 ± 5.60	0.654
RNFL 0-1 y	-2.35 ± 3.49	-1.60 ± 6.77	0.663
GCL 0-3 m	-1.25 ± 5.06	-3.00 ± 9.58	0.475
GCL 0-1 y	-2.00 ± 3.59	-2.45 ± 12.68	0.880
IPL 0-3 m	0.02 ± 10.01	-4.55 ± 7.18	0.094
IPL 0-1 y	-3.25 ± 6.60	-2.55 ± 9.12	0.783
INL 0-3 m	-0.65 ± 20.64	-8.05 ± 12.55	0.179
INL 0-1 y	-4.15 ± 12.26	-8.60 ± 15.30	0.317

* Independent samples t-test.
Values are presented as mean ± standard deviation and in microns.
TRT, total retinal thickness; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; 0-3 m, between baseline and the end of 3rd month; 0-1 y; between baseline and the end of 1 year.

Table 5: *Comparison of retinal layer thicknesses changes in R3 area at the end of 3rd month and of 1 year.*

	Ranibizumab n=20	Aflibercept n=20	p*
TRT 0-3 m	-32.02 ± 64.36	-50.35 ± 86.94	0.453
TRT 0-1 y	-44.64 ± 64.90	-50.95 ± 71.30	0.772
RNFL 0-3 m	-0.54 ± 4.01	-2.19 ± 5.56	0.289
RNFL 0-1 y	-1.12 ± 2.12	-2.00 ± 5.01	0.477
GCL 0-3 m	-1.24 ± 2.51	-2.03 ± 3.78	0.443
GCL 0-1 y	-2.10 ± 2.59	-2.40 ± 5.39	0.824
IPL 0-3 m	-0.19 ± 5.01	-3.37 ± 5.34	0.060
IPL 0-1 y	-1.14 ± 3.88	-2.48 ± 5.30	0.368
INL 0-3 m	-2.54 ± 8.96	-5.78 ± 8.71	0.255
INL 0-1 y	-3.62 ± 6.86	-6.25 ± 9.68	0.329

* Independent samples t-test.
Values are presented as mean ± standard deviation and in microns.
TRT, total retinal thickness; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; 0-3 m, between baseline and the end of 3rd month; 0-1 y; between baseline and the end of 1 year.

layer thicknesses from baseline to the end of 3rd month and of one year didn't show a statistically significant difference between groups in the R3 area (Table 5).

The mean total retina thickness change in the R3 area exhibited highly positive correlation with the mean RNFL thickness change in the R3 area ($r=0.608$, $p<0.001$) and a moderately positive correlation with the mean GCL thickness change in the R3 area ($r=0.443$, $p<0.05$) at the end of 1 year.

DISCUSSION

To our knowledge, our study is the first to compare the effects of ranibizumab and aflibercept on all inner retinal layers including INL. Several studies reported RNFL changes after anti-VEGF treatments. The outcomes of these studies varied from significant thinning[14,15] to no change[16-18] in RNFL after ranibizumab injections. Our study revealed clinically significant RNFL thinning only in the ranibizumab group after one year of treatment.

Heidelberg SD-OCT enabled us to measure GCL and IPL separately. GCL thinning was statistically significant after 3rd injection in both the ranibizumab and aflibercept groups. On the other hand, only the ranibizumab group showed statistically significant thinning after one year of follow-up. Concerning IPL, the statistically significant change was seen in the aflibercept group after 3rd injection, p-value in one year was equal to 0.05, very close to the clinical significance value in the same group. Beck et al[17] reported a significant decrease in GCL thickness after anti-VEGF injections in nAMD. Likewise, a significant thinning of GCIPL after anti-VEGF injections. was also shown by other studies.[14,18,19]

Our study reported INL changes after aflibercept injections in nAMD as well. This study revealed a significant decrease in thickness of INL in both ranibizumab and aflibercept groups after one year of follow-up. Inan et al analyzed long term changes after ranibizumab injections in nAMD in the same region of ETDRS grid as our study and they didn't find a statistically significant change in INL after one year of follow-up in the inner 3 mm parafoveal ring area of ETDRS grid in SD-OCT measurements.[18]

It is known that two high-resistance barriers to fluid flow through the retina are the synaptic portions of the outer plexiform layer and, inner plexiform layer.[20] In the case of nAMD which is the advanced form of AMD, it may be considered that these two barriers are broken down and consequently, fluid accumulation in inner retinal layers causes thickening of these layers. In a study done by Muftuoglu et al[21], nAMD patients had thicker mean RNFL, GCL, and IPL thicknesses within the fovea compared to healthy eyes. In our study, we found a highly positive correlation between the mean RNFL and the mean total retinal thickness change, a moderately positive correlation between the mean GCL and the mean total retinal thickness changes after one year, and the highest decreases in all these layers occurred after 3rd injection. Based on these findings, we can hypothesize that the thinning of all inner retinal layers in nAMD after anti-VEGF injections may be due to the resolution of fluid accumulated within these layers. Other theories explaining inner retinal thinning may be probable neurotoxic effecst of anti-VEGF agents or the transsynaptic degeneration of nAMD itself. [12] However, improvement in visual acuity as a result of anti-VEGF treatment in our study supports the first explanation about the therapeutic effect of anti-VEGF agents related to fluid resorption.

Besides evaluating the effects of anti-VEGF agents on inner retinal layers individually, a comparison of two different anti-VEGF agents, ranibizumab and aflibercept was the other purpose of the study. According to demographics and clinical characteristics, two groups possessed similar properties including gender, age, BCVA, IOP, and the mean number of injections. Baseline thicknesses of all studied layers in R1 and R3 areas were comparable and didn't show statistically significant differences between groups. Longitudinal analysis of changes in the total retina and inner retinal layers after anti-VEGF injections did not also reveal any statistically significant difference either after three months or one year in any studied area when we compared two groups. However, the aflibercept group responded faster in decreasing thicknesses in the total retina and all inner layers after 3rd. This difference became much smaller or reversed after one year except INL. BCVA was better after 3rd injection in aflibercept group but it wasn't statistically significant, compared with ranibizumab group. At the end of the first year, the difference of BCVA increase between groups diminished from 2.40 to 0.35 letters. Several studies comparing the ranibizumab and the aflibercept are consistent with our findings of visual acuity. Zhang et al[22] stated that aflibercept had comparable effects with ranibizumab for treatment-naive nAMD. Park et al[23] compared bevacizumab, ranibizumab, and aflibercept injections and found that they had similar responses for nAMD, although the number of injections of aflibercept was fewer than other anti-VEGF agents and aflibercept decreased pigment epithelial detachment more quickly. Bhandary et al[24] reported that neither ranibizumab nor aflibercept was superior to the other in terms of visual acuity outcomes and treatment frequency at three years for nAMD.

In conclusion, we demonstrated thinning of inner retinal layers as a consequence of one-year anti-VEGF treatment

in nAMD and no statistically significant difference between ranibizumab and aflibercept groups. We considered that the changes in inner retinal layers are mainly due to the therapeutic effects of anti-VEGF agents. The main limitation of our study is the small sample size. Further studies investigating the effects of anti-VEGF agents in lesion-free regions of the retina will corroborate the current study.

ACKNOWLEDGMENTS: None

DECLARATIONS:

Funding: This research received no specific grants from any funding agency in the public, commercial or not-for-profit sectors.

Author Contributions: Concept: N.D., B.K., Design: N.D., B.K., S.S., M.S., Data Collection or Processing: N.D., S.S., Analysis or Interpretation: N.D., B.K., S.S., M.S., Writing: N.D., B.K., S.S., M.S., Critical Revision: N.D., B.K., S.S., M.S.

Conflict of Interest: Demir N,none; Kayhan B, none; Sevincli S, none; Sonmez M, none.

Financial Disclosure: This study received no public or private support.

Ethics Committee Approval: This study was approved by the Ethical Committee of Saglık Bilimleri University and performed in adherence with the tenets of the Declaration of Helsinki (Approval number: 46418926-050.03.04).

REFERENCES

1. Klaver CC, Wolfs RC, Vingerling JR, et al. Age-specific prevalence and causes of blindness and visual impairment in an older population: the Rotterdam Study. *Arch Ophthalmol* 1998; 116: 653-658.
2. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. Eye diseases prevalence research group. *Arch Ophthalmol* 2004; 122(4): 564-572.
3. 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: 106-116.
4. Hoeben A, Landuyt B, Highley MS, et al. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* 2004; 56(4): 549-580.
5. Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. *J Biochem* 2013; 153(1): 13-19.
6. Solomon SD, Lindsley K, Vedula SS, et al. Anti-vascular endothelial growth factor for neovascular age-related macular degeneration. *Cochrane Database Syst Rev* 2019; 3(3): CD005139.
7. Gillies MC, Hunyor AP, Arnold JJ, et al. Effect of ranibizumab and aflibercept on best-corrected visual acuity in treat-and-extend for neovascular age-related macular degeneration: A randomized clinical trial. *JAMA Ophthalmol* 2019; 137(4): 372-379.
8. Foxton RH, Finkelstein A, Vijay S, et al. VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma. *Am J Pathol* 2013; 182(4): 1379-1390.
9. Saint-Geniez M, Maharaj AS, Walshe TE, et al. Endogenous VEGF is required for visual function: evidence for a survival role on müller cells and photoreceptors. *PLoS One* 2008; 3(11): e3554.
10. Cho HJ, Yoo SG, Kim HS, et al. Risk factors for geographic atrophy after intravitreal ranibizumab injections for retinal angiomatous proliferation. *Am J Ophthalmol* 2015; 159: 285-292.
11. Latzer P, Schlegel U and Theiss C. Morphological changes of cortical and hippocampal neurons after treatment with VEGF and bevacizumab. *CNS Neurosci Ther* 2016; 22(6): 440-450.
12. Park HY, Kim JH and Park CK. Neuronal cell death in the inner retina and the influence of vascular endothelial growth factor inhibition in a diabetic rat model. *Am J Pathol* 2014; 184(6): 1752-1762.
13. Muftuoglu IK, Ramkumar HL, Bartsch DU, et al. Quantitative analysis of the inner retinal layer thicknesses in age-related macular degeneration using corrected optical coherence tomography segmentation. *Retina* 2018; 38: 1478-1484.
14. Lee SW, Sim HE, Park JY, et al. Changes in inner retinal layer thickness in patients with exudative age-related macular degeneration during treatment with anti-vascular endothelial growth factor. *Medicine (Baltimore)* 2020; 99(17): e19955.
15. Martinez-de-la-Casa JM, Ruiz-Calvo A, Saenz-Frances F, et al. Retinal nerve fiber layer thickness changes in patients with age-related macular degeneration treated with intravitreal ranibizumab. *Invest Ophthalmol Vis Sci* 2012; 53(10): 6214-6218.
16. Demirel S, Batioğlu F, Özmert E, et al. The effect of multiple injections of ranibizumab on retinal nerve fiber layer thickness in patients with age-related macular degeneration. *Curr Eye Res* 2015; 40(1): 87-92.
17. Beck M, Munk MR, Ebneter A, et al. Retinal ganglion cell layer change in patients treated with anti-vascular endothelial growth factor for neovascular age-related macular degeneration. *Am J Ophthalmol* 2016; 167: 10-17.
18. Inan ÜÜ, Baysal Z and Inan S. Long-term changes in retinal layers in patients undergoing intravitreal ranibizumab for neovascular age-related macular degeneration: Retinal layers after anti-VEGF therapy. *Int Ophthalmol* 2019; 39(12): 2721-2730.
19. Kim SY, Yoon MH and Chin HS. Changes in the ganglion cell-inner plexiform layer after consecutive intravitreal injections of anti-vascular endothelial growth factor in age-related macular Degeneration Patients. *Korean J Ophthalmol* 2020; 34(1): 11-18.

20. Antcliff RJ, Hussain AA and Marshall J. Hydraulic conductivity of fixed retinal tissue after sequential excimer laser ablation: Barriers limiting fluid distribution and implications for cystoid macular edema. *Arch Ophthalmol* 2001; 119(4): 539-544.

21. Muftuoglu IK, Lin T and Freeman WR. Inner retinal thickening in newly diagnosed choroidal neovascularization. *Graefes Arch Clin Exp Ophthalmol* 2018; 256(11): 2035-2040.

22. Zhang Y, Chioreso C, Schweizer M, et al. Effects of aflibercept for neovascular age-related macular degeneration: A systematic review and meta-analysis of observational comparative studies. *Invest Ophthalmol Vis Sci* 2017; 58(13): 5616-5627.

23. Park DH, Sun HJ and Lee SJ. A comparison of responses to intravitreal bevacizumab, ranibizumab, or aflibercept injections for neovascular age-related macular degeneration. *Int Ophthalmol* 2017; 37(5): 1205-1214.

24. Bhandari S, Nguyen V, Arnold J, et al. Treatment outcomes of ranibizumab versus aflibercept for neovascular age-related macular degeneration: Data from the fight retinal blindness! registry. *Ophthalmology* 2020; 127(3): 369-376.