KJO
pISSN: 1011-8942 eISSN: 2092-9382

Korean J Ophthalmol 2020;34(1):11-18
https://doi.org/10.3341/kjo.2019.0081

Original Article

Changes in the Ganglion Cell-inner Plexiform Layer after Consecutive Intravitreal Injections of Anti-vascular Endothelial Growth Factor in Age-related Macular Degeneration Patients

Se Young Kim, Myung Hun Yoon, Hee Seung Chin

Department of Ophthalmology and Inha Vision Science Laboratory, Inha University Hospital, Inha University School of Medicine, Incheon, Korea

Purpose: To investigate the effect of intravitreal anti-vascular endothelial growth factor (VEGF) injections on ganglion cell-inner plexiform layer (GCIPL) thickness in patients with age-related macular degeneration (AMD).

Methods: This retrospective study included patients with continuous anti-VEGF treatment who were administered at least three consecutive injections for unilateral neovascular AMD. The GCIPL thickness of the study eyes was compared before and after treatment and with healthy fellow eyes using spectral-domain optical coherence tomography. We also evaluated best-corrected visual acuity, age, and intraocular pressure.

Results: In total, 96 eyes of 48 patients (14 females and 34 males; mean ± standard deviation [SD] age, 70.10 ± 8.89 years) with mean number of 6.29 (SD ± 3.76) anti-VEGF injections and a mean follow-up period of 24.93 months (SD ± 19.86) were included in the study. After three consecutive intravitreal injections of anti-VEGF, the mean GCIPL thickness was significantly reduced from 70.50 (SD ± 14.06) to 65.97 (SD ± 13.91) μm. Borderline or nonsignificant decrease was also observed in GCIPL thickness for each sector. At the end of the study, the mean GCIPL thickness was further reduced to 62.56 (SD ± 16.30) μm, and significant decreases were also observed in all other sectors compared with baseline.

Conclusions: It has been observed that GCIPL thickness can decrease with only three consecutive anti-VEGF injections as well as with long-term treatment in AMD patients.

Key Words: Aflibercept, Anti-vascular endothelial growth factor, Ganglion cell-inner plexiform layer, Macular degeneration, Ranibizumab

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the elderly population [1]. AMD causes functional deterioration of the central retina and its supporting structures, resulting in gradual reduction of visual acuity [2]. Neovascular age-related macular degeneration (nAMD), which affects 10% of AMD patients [3], is characterized by formation of choroidal neovascularization, pigment epithelial detachment, pigment epithelial tear, fibrovascular disciform scar, and vitreous hemorrhage [4]. It is well-established that vascular endothelial growth factor (VEGF) plays a key role in pathogenesis of neovascularization in nAMD [5]. Therefore, numerous drugs have been developed to inhibit

Received: July 4, 2019 *Final revision:* September 6, 2019
Accepted: September 11, 2019

Corresponding Author: Hee Seung Chin, MD, PhD. Department of Ophthalmology, Inha University Hospital, 27 Inhang-ro, Jung-gu, Incheon 22332, Korea. Tel: 82-32-890-2400, Fax: 82-32-890-2417, E-mail: hschin@inha.ac.kr

VEGF, and intravitreal injections of anti-VEGF are widely used as a standard treatment for nAMD. Intravitreal anti-VEGF injections are known to have excellent efficacy and be relatively safe for long-term use [6,7]. However, since VEGF plays an essential role in development and maintenance of ocular tissue, especially in the retina and choroid and in neuronal protection [8-10], inhibiting VEGF may theoretically have a negative impact on various tissues in the eye. Several studies have suggested that repeated intravitreal anti-VEGF injections lead to retinal pigment epithelium atrophy [11] as well as scleral thinning [12]. Furthermore, anti-VEGF is also reportedly toxic to retinal ganglion cells [13,14]. However, few studies have examined the effects of repeated intravitreal anti-VEGF injections on retinal ganglion cell layers in patients. In addition, as AMD persists for a long time, the natural course of the disease may result in decreased retinal ganglion cell layer [15]. Therefore, a prolonged observation period would make it difficult to identify the actual effect of anti-VEGF treatment alone on reduction of the retinal ganglion cell layer.

Therefore, we investigated the effects of anti-VEGF treatment on retinal ganglion cells in nAMD patients not only after long-term follow-up periods, but also in a short-duration follow-up period after only three consecutive monthly injections.

Materials and Methods

Participants

This study was carried out in accordance with the Declaration of Helsinki following approval of the relevant Institutional Review Board (INHAU 2017-10-001). This was a retrospective study conducted during the period of January 2012 to February 2018. We reviewed the medical records of nAMD patients who were identified as naïve at treatment baseline and who had been given at least three consecutive injections of intravitreal ranibizumab (Lucentis; Genentech, San Francisco, CA, USA) or aflibercept (Eylea; Regeneron, Tarrytown, NY, USA) at one-month intervals during the study period. All patients received the first three consecutive injections during the first three months and subsequently received injections using the Pro Re Nata protocol at the discretion of the physician. All patients were evaluated for corrected visual acuity, intraocular pressure, and optical coherence tomography results before and one month after the three consecutive intravitreal injections and at the end of the study; the healthy fellow eyes were similarly evaluated at baseline. Corrected visual acuity was measured using a Snellen chart and converted to logarithm of minimal angle of resolution (logMAR). Subjects were excluded if they had previous or current vitreoretinal diseases in the fellow eye, any vitreoretinal disease other than AMD in the study eye, media opacity that interfered with macular examination, a history of laser photocoagulation and ocular surgery other than cataract surgery, a history of intravitreal anti-VEGF injection prior to the study period, an elevating intraocular pressure exceeding 21 mmHg during the study period, or high myopia (spherical equivalent ≤-6.0 diopters). Subjects with a history of dementia, multiple sclerosis, or glaucoma that could impact the ganglion cell-inner plexiform layer (GCIPL) were also excluded.

Optical coherence tomography analysis

The retinal ganglion cell layers were measured using spectral-domain optical coherence tomography (OCT) (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA, USA). One macular scan (macular cube 514 × 128 protocol) was obtained for each subject; the macular protocol also included a ganglion cell analysis, which detects and measures the thickness of the macular GCIPL within a 14.13-mm^2 elliptical annulus area centered on the fovea. The GCIPL thickness measurements were analyzed as mean, minimum, and sectoral (e.g., superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal) values. All parameters were measured automatically using the Cirrus internal macular thickness analysis algorithm. In this study, only good-quality OCT images with a signal strength greater than 6 were analyzed. All OCT scans were performed by a single certified technician. Two independent researchers (YMH and KSY), blinded to the results of the study, analyzed the OCT scans; subjects were excluded if the GCIPL layers automatically segmented by the Cirrus program were inaccurate due to severe macular edema, submacular hemorrhage, submacular fluid collection, or macular distortion.

Statistical analysis

Statistical analysis was performed using the PASW Statistics ver. 18 (SPSS Inc., Chicago, IL, USA). Comparisons between two independent groups were performed using Student's *t*-test, and comparisons between two paired measurements were performed using paired *t*-test. Statistical significance was defined as a *p*-value less than 0.05.

Results

Clinical features before treatment

Of the 184 subjects identified during the study period, 48 who met the inclusion criteria were enrolled in the study; the remaining either met the exclusion criteria or were excluded because OCT segmentation of GCIP was not accurate. In total, 48 subject eyes and 48 healthy fellow eyes of 48 patients were analyzed. The subjects included 34 males and 14 females; of these, 25 patients were given ranibizumab, and 23 patients received aflibercept. The mean age was 70.10 ± 8.89 years, ranging from 54 to 92 years, and laterality was evenly distributed, in 24 right eyes and 24 left eyes. The mean corrected visual acuity was logMAR 0.70 ± 0.41 in the study eyes and 0.19 ± 0.24 in fellow eyes ($p < 0.001$). The mean intraocular pressure was 15.27 ± 3.25 mmHg in the study eyes and 15.66 ± 3.17 mmHg in fellow eyes ($p = 0.548$). The mean and minimum GCIPL thickness values were 70.50 ± 14.06 and 45.64 ± 19.96 μm, respectively, in the study eyes and 76.31 ± 11.52 and 66.66 ± 18.61 μm in the fellow eyes. The mean and minimal GCIPL thickness values of study eyes were significantly lower than those of fellow eyes ($p = 0.029$ and $p < 0.001$, respectively). The GCIPL thickness the superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal sectors was 69.58 ± 15.73, 69.50 ± 18.90, 74.18 ± 19.60, 72.58 ± 22.25, 67.75 ± 19.48, and 69.27 ± 18.72 μm, respectively, in the study eyes and 75.04 ± 12.83, 76.00 ± 14.11, 78.37 ± 11.71, 77.85 ± 10.75, 74.47 ± 12.71, and 76.52 ± 12.87 μm in

Table 1. Baseline characteristics of study patients

Characteristics	Study eye	Fellow eye	*p*-value*
Age (yr)	70.10 ± 8.89	70.10 ± 8.89	
Sex, female : male	14 : 34	14 : 34	
Laterality, right : left	24 : 24	24 : 24	
No. of injections after loading treatment	3	0 ± 0.00	0.000
Total number of injections at the end of the study	6.29 ± 3.76	0 ± 0.00	0.000
Follow-up time (mon)	24.93 ± 19.86		
BCVA (logMAR)	0.70 ± 0.41	0.19 ± 0.24	<0.001
Intraocular pressure (mmHg)	15.27 ± 3.25	15.66 ± 3.17	0.548
Spherical equivalent (diopters)	0.49 ± 1.54	0.64 ± 1.49	0.617
Ganglion cell-inner plexiform layer (μm)			
Average	70.50 ± 14.06	76.31 ± 11.52	0.029
Minimum	45.64 ± 19.96	66.66 ± 18.61	<0.001
Superotemporal	69.58 ± 15.73	75.04 ± 12.83	0.066
Superior	69.50 ± 18.90	76.00 ± 14.11	0.059
Superonasal	74.18 ± 19.60	78.37 ± 11.71	0.207
Inferonasal	72.58 ± 22.25	77.85 ± 10.75	0.143
Inferior	67.75 ± 19.48	74.47 ± 12.71	0.048
Inferotemporal	69.27 ± 18.72	76.52 ± 12.87	0.029

Values are presented as mean ± standard deviation.
BCVA = best-corrected visual acuity; logMAR = logarithm of minimal angle of resolution.
*Independent samples *t*-test.

fellow eyes. Inferior and inferotemporal sectors were significantly lower (p = 0.048 and 0.029, respectively) in subject eyes compared to fellow eyes, with no significant differences in the other sectors (all $p > 0.05$) (Table 1). No statistically significant difference was observed with respect to age, corrected visual acuity, intraocular pressure, or GCIPL thickness upon comparing the ranibizumab group and the aflibercept group.

GCIPL thickness change after three consecutive monthly injections

The GCIPL thickness as measured by OCT, corrected visual acuity, and intraocular pressure was evaluated before treatment, at one month after the three consecutive monthly loading injections (ranibizumab or aflibercept), and at the end of the study period in all 48 subject eyes with AMD. Analysis revealed that corrected visual acuity improved significantly from logMAR 0.70 ± 0.41 before treatment to 0.57 ± 0.44 after loading treatment (p = 0.001). The intraocular pressure decreased from 15.27 ± 3.25 to 15.04 ± 2.91 mmHg but was not statistically significant (p = 0.575). Separately, mean GCIPL thickness significantly decreased from 70.50 ± 14.06 to 65.97 ± 13.91 μm (p = 0.004) (Fig. 1). Minimal GCIPL thickness decreased from 45.64 ± 19.96 to 42.66 ± 22.41 μm, but this was not a significant change (p = 0.237). The thickness of each sector was as follows: for the superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal sectors, values were 69.58 ± 15.73, 69.50 ± 18.90, 74.18 ± 19.60, 72.58 ± 22.25, 67.75 ± 19.48, and 69.27 ± 18.72 μm, respectively, before treatment and 64.52 ± 17.39, 64.31 ± 18.16, 70.83 ± 16.20, 67.25 ± 18.33, 62.37 ± 19.44, and 66.29 ± 19.28 μm after treatment. We observed that GCIPL thickness was lower in all sectors after treatment than before treatment, but the change was statistically significant only in the superotemporal (p = 0.011) and superior sectors (p = 0.045) (Table 2). Fig. 2A-2D presents a case example for these findings.

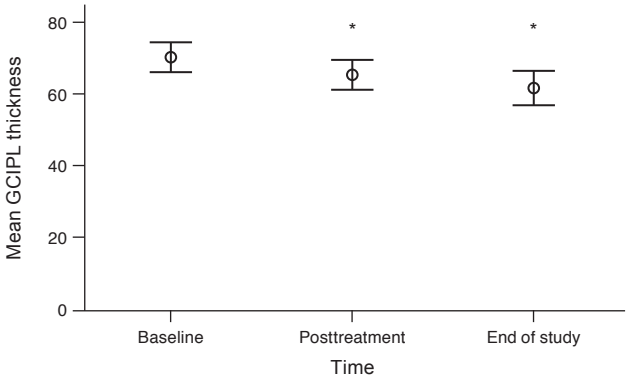

Fig. 1. Representative map of mean ganglion cell-inner plexiform layer (GCIPL) at baseline, after three consecutive monthly loading treatments with anti-vascular endothelial growth factor for neovascular age-related macular degeneration (posttreatment), and at study end (end of study). The graph shows that mean GCIPL thickness decreases from baseline to after loading treatment and then toward the end of the study. $^{*}p < 0.05$.

GCIPL thickness change at the end of the study

At the end of the study, after a mean follow-up period of 24.93 months (SD ± 19.86), corrected visual acuity improved significantly from logMAR 0.70 ± 0.41 before treatment to 0.56 ± 0.43 (p = 0.005). Intraocular pressure was not significantly changed over the study period, from 15.27 ± 3.25 to 15.00 ± 2.93 mmHg (p = 0.522). Mean GCIPL thickness significantly decreased from 70.50 ± 14.06 to 62.56 ± 16.30 μm ($p < 0.001$) (Fig. 1), while minimal GCIPL thickness decreased from 45.64 ± 19.96 to 38.70 ± 23.47 μm (p = 0.048). The thickness of each sector assessed was as follows: for the superotemporal, superior, superonasal, inferonasal, inferior, and inferotemporal sectors, the values were 69.58 ± 15.73, 69.50 ± 18.90, 74.18 ± 19.60, 72.58 ± 22.25, 67.75 ± 19.48, and 69.27 ± 18.72 μm, respectively, before treatment and 62.22 ± 19.99, 60.62 ± 19.78, 67.79 ± 18.75, 64.64 ± 19.34, 56.08 ± 21.29, and 59.83 ± 21.40 μm after treatment. The GCIPL thickness was significantly lower in all sectors at the end of the study period than before treatment (p = 0.003, 0.002, 0.033, 0.023, 0.001, and 0.001, respectively) (Table 2).

Discussion

Since treatment of nAMD with intravitreal anti-VEGF injection is recognized to have an excellent therapeutic outcome compared with previous conventional treatments, many patients have been treated with long-term repeated injections. However, despite the therapeutic efficacy of anti-VEGF drugs, the corresponding side effects are unclear. As described above, since VEGF plays an important role in maintenance of retinal and choroidal tissue and also has

Table 2. Changes in GCIPL before and after treatment

	Pretreatment*	Posttreatment*	End of the study	*p*-value†	*p*-value‡
BCVA (logMAR)	0.70 ± 0.41	0.57 ± 0.44	0.56 ± 0.43	0.001	0.005
IOP (mmHg)	15.27 ± 3.25	15.04 ± 2.91	15.00 ± 2.93	0.576	0.522
GCIPL (μm)					
Average	70.50 ± 14.06	65.97 ± 13.91	62.56 ± 16.30	0.004	0.000
Minimum	45.64 ± 19.96	42.66 ± 22.41	38.70 ± 23.47	0.237	0.048
Superotemporal	69.58 ± 15.73	64.52 ± 17.39	62.22 ± 19.99	0.011	0.003
Superior	69.50 ± 18.90	64.31 ± 18.16	60.62 ± 19.78	0.045	0.002
Superonasal	74.18 ± 19.60	70.83 ± 16.20	67.79 ± 18.75	0.181	0.033
Inferonasal	72.58 ± 22.25	67.25 ± 18.33	64.64 ± 19.34	0.061	0.023
Inferior	67.75 ± 19.48	62.37 ± 19.44	56.08 ± 21.29	0.076	0.001
Inferotemporal	69.27 ± 18.72	66.29 ± 19.28	59.83 ± 21.40	0.124	0.001

Values are presented as mean ± standard deviation.
GCIPL = ganglion cell-inner plexiform layer; BCVA = best-corrected visual acuity; logMAR = logarithm of minimal angle of resolution; IOP = intraocular pressure.
*Treatment means the first three consecutive monthly loading injections; †Comparing pre-treatment with posttreatment, paired samples *t*-test; ‡Comparing pretreatment with last follow-up, paired samples *t*-test.

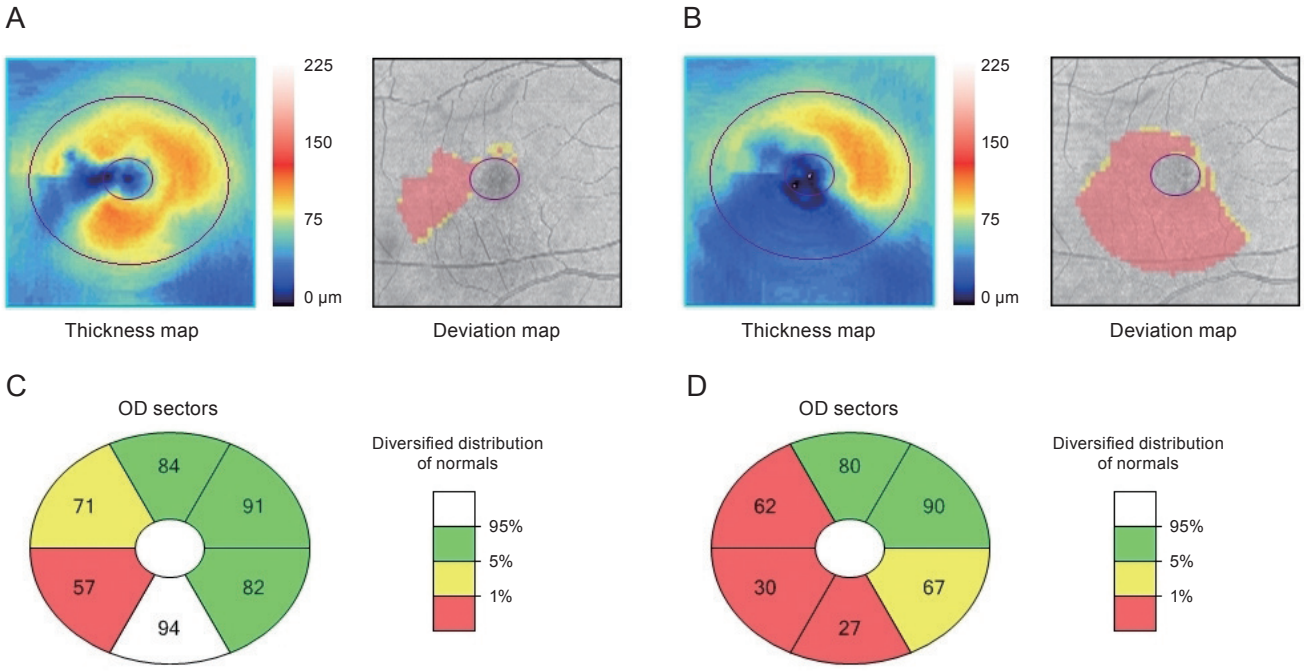

Fig. 2. Case example of an age-related macular degeneration patient who was treated with anti-vascular endothelial growth factor therapy. A 60-year-old patient was administered three consecutive monthly intravitreal injections of aflibercept. A representative map of ganglion cell-inner plexiform layer thickness (A) at baseline and (B) after treatment. Each sectoral measurement of ganglion cell-inner plexiform layer thickness was also demonstrated (C) at baseline and (D) after treatment. OD = oculus dexter.

neuroprotective effects [8-10], artificial blockage of VEGF may result in intraocular complications. In fact, several studies have reported that repeated anti-VEGF treatment leads to atrophy of various ocular tissues such as the sclera [12] and retinal pigment epithelium [11].

In a review of the literature investigating the effects of anti-VEGF on retinal ganglion cells, a laboratory study showed that VEGF protects retinal ganglion cells from ox-

idative stress, and this protective effect is eliminated by treatment with bevacizumab [16]. A similar study reported that survival of retinal ganglion cells under oxidative stress decreased with increasing concentration of bevacizumab administration [14]. An animal study further suggested that retinal ganglion cells were severely damaged when VEGF was blocked [10], while another study indicated no changes in the retinal ganglion cell layer after VEGF inhibition [17]. Previous clinical studies of retinal alterations after intravitreal injections of anti-VEGF mostly reported changes in the retinal nerve fiber layer (RNFL) assessed using OCT. One study found that long-term treatment with anti-VEGF caused no significant change in RNFL thickness in patients with AMD [18]. Conversely, another report found that RNFL thickness was significantly decreased at 12 weeks after treatment with two intravitreal bevacizumab injections in patients with wet-type AMD, while there was no significant difference at 24 weeks compared with baseline [19]. In a study of various diseases including AMD, diabetic retinopathy, and retinal vein occlusion, anti-VEGF treatment had no significant effect on RNFL thickness [20]. Hence, it is difficult to clearly conclude the relationship between intravitreal anti-VEGF treatment and changes in RNFL thickness. However, the retinal ganglion cell layer in glaucoma patients is more sensitive to changes in the visual field than alterations in the peripapillary retinal nerve fiber layer [21,22]. Therefore, a study evaluating changes in the retinal ganglion cell layer after intravitreal anti-VEGF injection therapy may be more meaningful, although there is only one such study published to date. According to that study, retinal ganglion cell layer thickness significantly decreased, with no significant change in RNFL thickness after an average of 31.5 anti-VEGF injection treatments over a mean of 45.3 months [23]. However, it is difficult to distinguish the cause of decrease in retinal ganglion cell layer thickness; it could either be a natural course associated with progression of AMD over the long-term follow-up, an effect of anti-VEGF drugs, or an intraocular pressure spike after intravitreal injection. Differing numbers and intervals of intravitreal injections also interfere with determination of the impact of anti-VEGF treatment on the retinal ganglion cell layer. Hence, we believe the results of the current study are meaningful, since the study assesses three consecutive anti-VEGF loading treatments commonly used in real-world clinical practice, and all subjects were administered the same number of injections (three) and participated in the same observation period after baseline (3 months), thereby controlling variables that would affect the outcome. Also, a short observation period could reduce the effect of ganglion cell layer decrease due to long-term AMD progression rather than anti-VEGF treatment. We effectively evaluated the fundamental effects of anti-VEGF treatment by standardizing the treatment conditions and maintaining a high concentration of intraocular drug by administering an intensive treatment over a short duration.

To our knowledge, this study was the first to investigate the change in GCIPL after anti-VEGF treatment using the Cirrus OCT system. The Cirrus OCT system may experience segmentation errors during measurement of GCIPL when macular lesions are present [24,25]. A previous study using the Cirrus HD-OCT system reported a larger number of scanning artifacts in eyes with wet AMD (64.4%) compared to those with dry AMD (20.5%) [25]. The authors suggested that morphologic features such as fluid between the retina and the retinal pigment epithelium or fluid within the retina led to misidentification of retinal boundaries, resulting in segmentation errors [25]. Recently, there have been several studies on automatic segmentation errors that arise while using various OCT devices [26-28]. Kim et al. [26] reported the frequency of segmentation errors with the Spectralis OCT system (Heidelberg Engineering, Heidelberg, Germany) and found clinically significant errors greater than 50 μm in 17 eyes (6.4%), 13 (43.3%) of which had wet AMD. Ho et al. [27] evaluated different OCT device systems among patients with various retinal diseases and concluded that the Cirrus HD-OCT system produced the lowest percentage of any type of artifact in patients with wet AMD. Hence, to improve accuracy, all cases with segmentation errors were excluded from the analysis, and only cases with good segmentation outcomes were included. In our study, 41 (22%) of the 184 patients were excluded because of inaccurate automatic segmentation.

There are some limitations to this study, including primarily its retrospective nature. Also, due to the small sample size, the statistical power was weak as segmentation error and other exclusion criteria were strictly applied, leading to a sacrifice of statistical power but improving the accuracy of the results. Consequently, a statistically significant decrease was observed only in the mean, superotemporal, and superior GCIPL thicknesses after loading treat-

ment. However, even though the results were not statistically significant, a decrease in thickness was observed in all other GCIPL regions. Furthermore, the declining trend of GCIPL after anti-VEGF treatment became more apparent toward the end of the study, when statistically significant decreases in all measurements of GCIPL were detected. We therefore hypothesize that statistical significance could be obtained by increasing the sample size. Additionally, the mean, minimum, inferior, and inferotemporal GCIPL values were slightly lower in the study eyes compared to fellow eyes before treatment, which is similar to the results of other studies that report thinner retinal ganglion cell layer in nAMD than in healthy controls [15]. Although this study is meaningful as effects were evaluated in a short-duration follow-up period after three consecutive injections, the short observation period compared with those of other studies is still a major limitation. In one study, RNFL (not GCIPL) thickness was significantly decreased at 12 weeks after two intravitreal bevacizumab injections in patients with AMD but was not significant at 24 weeks [19]. Contrarily, our study showed a decrease in GCIPL not only after the short term, but after an average of 24.93 ± 19.86 months. However, we are still unable to predict how GCIPL thickness will change after a longer period. Further research is required to compare the results obtained after a longer duration in these patients.

In conclusion, although anti-VEGF injection therapy is relatively safe in AMD patients, careful observation is needed to consider the possibility of GCIPL reduction with only three doses of loading treatment. Furthermore, we should also consider the possibility of GCIPL thickness reduction with a larger number of injections over a long period. Therefore, in long-term treatment of intravitreal anti-VEGF injections for nAMD patients, especially those with glaucoma, it would be beneficial to aim efforts toward reducing total amount of medication by tailoring individualized therapies using various methods such as pro-re-nata or treat-and-extend.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by Inha University Research Grant, Incheon, Korea.

References

1. Sobrin L, Seddon JM. Nature and nurture- genes and environment- predict onset and progression of macular degeneration. *Prog Retin Eye Res* 2014;40:1-15.
2. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 1995;39:367-74.
3. Ferris FL 3rd, Fine SL, Hyman L. Age-related macular degeneration and blindness due to neovascular maculopathy. *Arch Ophthalmol* 1984;102:1640-2.
4. Bressler NM, Bressler SB, Fine SL. Age-related macular degeneration. *Surv Ophthalmol* 1988;32:375-413.
5. Ambati J, Ambati BK, Yoo SH, et al. Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. *Surv Ophthalmol* 2003;48:257-93.
6. CATT Research Group, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med* 2011;364:1897-908.
7. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012;119:1388-98.
8. Blaauwgeers HG, Holtkamp GM, Rutten H, et al. Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. *Am J Pathol* 1999;155:421-8.
9. Marneros AG, Fan J, Yokoyama Y, et al. Vascular endothelial growth factor expression in the retinal pigment epithelium is essential for choriocapillaris development and visual function. *Am J Pathol* 2005;167:1451-9.
10. Nishijima K, Ng YS, Zhong L, et al. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. *Am J Pathol* 2007;171:53-67.

11. 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;159:285-92.
12. Zinkernagel MS, Schorno P, Ebneter A, Wolf S. Scleral thinning after repeated intravitreal injections of antivascular endothelial growth factor agents in the same quadrant. *Invest Ophthalmol Vis Sci* 2015;56:1894-900.
13. 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;182:1379-90.
14. Lee JM, Bae HW, Lee SY, et al. Effect of anti-vascular endothelial growth factor antibody on the survival of cultured retinal ganglion cells. *Korean J Ophthalmol* 2017;31:360-5.
15. Zucchiatti I, Parodi MB, Pierro L, et al. Macular ganglion cell complex and retinal nerve fiber layer comparison in different stages of age-related macular degeneration. *Am J Ophthalmol* 2015;160:602-7.
16. Brar VS, Sharma RK, Murthy RK, Chalam KV. Bevacizumab neutralizes the protective effect of vascular endothelial growth factor on retinal ganglion cells. *Mol Vis* 2010;16:1848-53.
17. Cheng CK, Peng PH, Tien LT, et al. Bevacizumab is not toxic to retinal ganglion cells after repeated intravitreal injection. *Retina* 2009;29:306-12.
18. Horsley MB, Mandava N, Maycotte MA, Kahook MY. Retinal nerve fiber layer thickness in patients receiving chronic anti-vascular endothelial growth factor therapy. *Am J Ophthalmol* 2010;150:558-61.
19. Entezari M, Ramezani A, Yaseri M. Changes in retinal nerve fiber layer thickness after two intravitreal bevacizumab injections for wet type age-related macular degeneration. *J Ophthalmic Vis Res* 2014;9:449-52.
20. Shin HJ, Shin KC, Chung H, Kim HC. Change of retinal nerve fiber layer thickness in various retinal diseases treated with multiple intravitreal antivascular endothelial growth factor. *Invest Ophthalmol Vis Sci* 2014;55:2403-11.
21. Shin HY, Park HY, Jung KI, Park CK. Comparative study of macular ganglion cell-inner plexiform layer and peripapillary retinal nerve fiber layer measurement: structure-function analysis. *Invest Ophthalmol Vis Sci* 2013;54:7344-53.
22. Ohkubo S, Higashide T, Udagawa S, et al. Focal relationship between structure and function within the central 10 degrees in glaucoma. *Invest Ophthalmol Vis Sci* 2014;55:5269-77.
23. 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;167:10-7.
24. Lee HJ, Kim MS, Jo YJ, Kim JY. Ganglion cell-inner plexiform layer thickness in retinal diseases: repeatability study of spectral-domain optical coherence tomography. *Am J Ophthalmol* 2015;160:283-9.
25. Alshareef RA, Goud A, Mikhail M, et al. Segmentation errors in macular ganglion cell analysis as determined by optical coherence tomography in eyes with macular pathology. *Int J Retina Vitreous* 2017;3:25.
26. Kim HJ, Chung H, Kim HC. Artifacts associated with spectral-domain optical coherence tomography. *J Korean Ophthalmol Soc* 2011;52:943-51.
27. Ho J, Sull AC, Vuong LN, et al. Assessment of artifacts and reproducibility across spectral- and time-domain optical coherence tomography devices. *Ophthalmology* 2009;116:1960-70.
28. Patel PJ, Chen FK, da Cruz L, Tufail A. Segmentation error in Stratus optical coherence tomography for neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2009;50:399-404.