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Macular ganglion cell complex changes in eyes treated with aflibercept for neovascular age-related macular degeneration

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ABSTRACT

Background: To evaluate the effect of intravitreal aflibercept injection on macular ganglion cell complex (GCC) in patients with neovascular age-related macular degeneration (nAMD).
Methods: In total, 36 eyes of 36 treatment-naïve patients with nAMD (18 female and 18 male) and 36 eyes of 36 healthy subjects (20 female and 16 male) as controls were included in this retrospective study. Spectral-domain optical coherence tomography images were evaluated after each injection for 3 months and at 12 months. Mean GCC thickness of the center, inner ring, and outer ring of the ETDRS grid was automatically quantified.
Results: Mean foveal thickness was statistically significantly decreased at 3 months and at 12 months compared with baseline in the patient group. Ganglion cell layer thickness in the center was statistically significantly decreased in eyes with nAMD at baseline. There was a statistically significant decrease for mean retinal nerve fiber layer, ganglion cell layer, and inner plexiform layer thickness at 12 months compared with baseline.
Conclusions: Macular GCC thickness was decreased after intravitreal aflibercept injection in patients with nAMD, in particular at 12 months.

1. Introduction

Age-related macular degeneration (AMD) is the most common cause of severe vision loss in elderly people in industrialized countries [1]. Neovascular AMD (nAMD) is characterized by the growth of new blood vessels due to an abnormal release of vascular endothelial growth factor-A (VEGF-A). The use of antiangiogenic drugs (anti-VEGF-A antibodies), which are administered by intravitreal injection, is one of the most effective treatments for nAMD [2-5].

Aflibercept is a fusion protein that binds with domains from native VEGF receptors, such as VEGF-A, VEGF-B, and placental growth factors 1 and 2, with high affinity [6]. David et al. demonstrated that aflibercept suppresses choroidal neovascularization in patients with nAMD with excellent visual outcomes [6]. In many patients with nAMD, anti-VEGF agents need to be continuously administered for many years to persistently suppress disease activity and maintain visual acuity.

Despite the beneficial effects of anti-VEGF treatment, the side effects of long-term use remain unclear and are a matter of ongoing controversy. There is evidence that repeated long-term intravitreal anti-VEGF treatment may accelerate atrophy of intraocular tissues. Atrophy of retinal pigment epithelium and scleral thinning have been reported [7, 8]. Several studies have evaluated the effect of intravitreal anti-VEGF injections on the peripapillary retinal nerve fiber layer (RNFL). Certain studies on animals have reported severe damage to retinal ganglion cells following treatment with anti-VEGF agents, while others have found no significant changes in the retinal ganglion cell layer (GCL) after the VEGF receptor blockade [9,10].

The aim of our study was to evaluate the effect of intravitreal aflibercept injection treatment on the macular ganglion cell complex (GCC), including the GCL, RNFL, and inner plexiform layer (IPL), in patients with nAMD.

2. Materials and methods

Treatment-naïve patients who were followed for at least 12 months for nAMD and received intravitreal aflibercept (2 mg/0.05 ml) (Eylea; Regeneron, Tarrytown, NY, USA, and Bayer, Leverkusen, Germany) injection were included in this retrospective study. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee.

The control group comprised randomly selected, age- and gender-matched healthy subjects who attended the outpatient clinic for

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routine ocular examination. The inclusion criteria were as follows: best-corrected visual acuity (BCVA) of 20/20 or more, refractive error between +2 D and −2 D spherical equivalent, intraocular pressure (IOP) lower than 21 mmHg, clear media, normal fundus and optic disk appearance, no previous ocular surgery, and no history of ocular and systemic diseases (diabetes, hypertension, and neurological diseases).

All participants underwent a complete ophthalmic examination, including the measurement of BCVA in logMAR units, slit-lamp biomicroscopy, fundoscopy, and spectral-domain optical coherence tomography (SD-OCT) imaging (Spectralis®, Heidelberg Engineering Inc., Heidelberg, Germany). SD-OCT images of the patient group were assessed after each injection for 3 months and repeated at 12 months.

The diagnosis of nAMD was based on fundoscopy, fluorescein angiography, and SD-OCT findings. Subjects with the presence of protruded orange-red elevated lesions resembling polypoidal choroidal vasculopathy on the fundoscopic exam and/or those with polypoidal vasculopathy findings in SD-OCT were excluded. Other criteria for exclusion were determined as the presence of ocular hypertension (IOP $>$ 21 mmHg), glaucoma, additional retinal disorders, such as vascular occlusion, diabetic retinopathy, hypertensive retinopathy, central serous chorioretinopathy, previous intraocular surgery, or previous retinal laser photocoagulation.

SD-OCT images were captured between 9:00 am and 12:00 pm by the same technician after dilation of the pupils with 0.5% tropicamide solution. Only scans with a signal strength of at least six or above and good reliability were included in the analysis.

All participants were examined using the standard posterior pole and RNFL protocols of the OCT. Images were acquired to obtain perifoveal volumetric retinal scans comprising 61 single lines of 15 frames centered at the fovea. Segmentation of each retinal layer was checked by a blinded physician using Heidelberg Eye Explorer version 1.9.10.0 (Heidelberg Engineering).

The macular area was divided into 9 regions as defined by the The Early Treatment of Diabetic Retinopathy Study (ETDRS) circle: 3 concentric circles centered at the fovea with diameters of 1 mm (center), 3 mm (inner circle), and 6 mm (outer circle) and 2 diagonal lines that divided the inner and outer circles into 4 regions each: superior, nasal, inferior, and temporal. The thicknesses of the RNFL, GCL, and IPL of the center and the inner and outer rings were measured and recorded. GCC was also measured in the analysis and corresponded to the combination of RNFL, GCL, and IPL, respectively.

Statistical analysis was conducted with SPSS 11.5 (SPSS Inc., Chicago, IL, USA). The student's *t*-test for independent data and for paired data was used to compare parameters assessed before and after each injection. All quantitative values were expressed as mean ± standard deviations. A p-value below 0.05 was set for statistical significance.

3. Results

In total, 36 eyes of 36 patients with treatment-naïve nAMD (18 female and 18 male, mean age 64.2 ± 3.4 years) and 36 eyes of 36 healthy controls (20 female and 16 male, mean age 62.1 ± 2.9 years) were included in the study. The mean number of intravitreal injections during the 12-month follow-up was 8.2 ± 0.98, and the mean number of visits was 11.02 ± 1.2.

The mean BCVA was 0.42 logMAR units at the baseline, 0.22 logMAR units after 3 doses of intravitreal injection ($p = 0.016$), and 0.19 logMAR units at 12 months. The increase in BCVA was statistically significant when compared to the baseline ($p = 0.001$). There was no significant increase in IOP after intravitreal injections in the patient group.

The mean foveal thickness was statistically significantly decreased in the patient group ($p < 0.001$); it was statistically significantly decreased at 3 months and 12 months when compared to the baseline ($p = 0.001$ and $p < 0.001$, respectively) and statistically significantly decreased after the first and second injections when compared to the baseline ($p < 0.001$ and $p < 0.001$, respectively). After the first, second, and third injections, the mean foveal thickness was statistically significantly different when compared to the controls ($p < 0.001$, $p = 0.001$, and $p < 0.001$, respectively). At 12 months, the mean foveal thickness was statistically significantly different when compared to the controls ($p < 0.001$).

In the center of the ETDRS grid, at the baseline, the difference between the patient and control groups for the mean RNFL and IPL thicknesses was not statistically significant ($p = 1.0$ and $p = 0.093$, respectively). GCL thickness was statistically significantly decreased in the patient group ($p < 0.001$).

After the first injection, the mean RNFL and GCL thicknesses did not statistically significantly differ when compared with the baseline ($p = 0.600$ and $p = 0.705$, respectively). The mean RNFL thickness was also not statistically significantly different when compared with the controls ($p = 0.108$), while the mean GCL thickness showed a statistically significant difference when compared to the controls ($p < 0.001$). The mean IPL thickness was not statistically significantly different when compared with the baseline ($p = 0.883$) but was statistically significantly different when compared to the controls ($p = 0.009$).

After the second injection, the mean RNFL, GCL, and IPL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.197$, $p = 0.557$, and $p = 0.506$, respectively). The difference was statistically significant when compared to the controls ($p = 0.017$, $p < 0.001$, and $p = 0.001$, respectively).

After the third injection, the mean RNFL and GCL thicknesses did not show a statistically significant difference when compared with the baseline ($p = 0.07$ and $p = 0.265$, respectively). There was a statistically significant difference when compared to the control group ($p = 0.004$ and $p < 0.001$, respectively). The mean IPL thickness was not statistically significantly different when compared with the baseline ($p = 0.086$), but the difference was statistically significant with respect to the controls ($p < 0.001$).

At 12 months, the mean RNFL and GCL thicknesses were statistically significantly decreased when compared to the baseline and control eyes ($p < 0.001$, $p = 0.004$ and $p < 0.001$, $p < 0.001$, respectively). There was also a statistically significant decrease in IPL thickness at 12 months when compared to the baseline ($p = 0.001$).

In the parafoveal region of the ETDRS grid, at the baseline, the mean RNFL and IPL thicknesses of the patient group were statistically significantly different in comparison to the control group ($p = 0.015$ and $p = 0.005$, respectively). The mean GCL thickness of the patient group did not show a statistically significant difference when compared with the controls ($p = 0.281$).

After the first injection, the mean RNFL and GCL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.740$ and $p = 0.874$, respectively). The mean RNFL thickness showed a statistically significant difference when compared to the controls ($p = 0.004$). The mean GCL thickness was not statistically significantly different when compared with the controls ($p = 0.219$). The mean IPL thickness was not statistically significantly different in the treated eyes when compared with the baseline ($p = 0.649$), but the difference was statistically significantly different when compared to the controls ($p = 0.019$).

After the second injection, the mean RNFL and GCL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.242$ and $p = 0.314$, respectively). The mean RNFL thickness was statistically significantly different when compared to the controls ($p = 0.003$). The mean GCL thickness did not show a statistically significant difference when compared with the controls ($p = 0.194$). The mean IPL thickness was not statistically significantly different when compared with the baseline ($p = 0.408$), but the difference was statistically significantly different when compared to the controls (p ˂ 0.001).

After the third injection, the mean RNFL and GCL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.515$ and $p = 0.233$, respectively), but they were statistically significantly different when compared to the controls ($p = 0.002$ and p

Table 1
Mean foveal thickness, RNFL, GCL, and IPL thickness in study groups.

	Mean±SD Patients ($n = 36$)						p-values Comparisons								
	Baseline	After 1st injection	After 2nd injection	After 3rd injection	12th month	Controls ($n = 36$)	Baseline vs After 1st injection	Baseline vs After 2nd injection	Baseline vs After 3rd injection	Baseline vs 12th month	Baseline vs Controls	Controls vs After 1st injection	Controls vs After 2nd injection	Controls vs After 3rd injection	Controls vs 12th month
Foveal thickness (μm)	348.3 ± 51.4	301.2 ± 41.2	298.2 ± 40.6	294.4 ± 39.8	284.2 ± 27.4	212.2 ± 18.8	< 0.001*	< 0.001*	0.001*	< 0.001*	< 0.001*	< 0.001*	0.001*	< 0.001*	< 0.001*
Central															
RNFL thickness (μm)	13.9 ± 1.8	13.7 ± 1.6	13.4 ± 1.7	13.1 ± 2.1	10.7 ± 1.4	14.4 ± 1.5	0.600	0.197	0.070	< 0.001*	1.000	0.108	0.017*	0.004*	< 0.001*
GCL thickness (μm)	12.4 ± 2.3	12.2 ± 2.1	12.1 ± 1.9	11.8 ± 2.04	10.9 ± 1.7	14.8 ± 2.1	0.705	0.557	0.265	0.004*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
IPL thickness (μm)	17.9 ± 4.1	17.7 ± 4.2	17.2 ± 3.9	16.2 ± 3.7	14.8 ± 3.6	20.1 ± 2.7	0.883	0.506	0.086	0.001*	0.093	0.009*	0.001*	< 0.001*	0.001*
Inner Ring (Parafoveal)															
RNFL thickness (μm)	21.1 ± 2.7	20.9 ± 2.4	20.9 ± 2.2	20.7 ± 2.5	19.8 ± 2.4	22.7 ± 2.3	0.740	0.242	0.515	0.037*	0.015*	0.004*	0.003*	0.002*	< 0.001*
GCL thickness (μm)	48.1 ± 7.1	47.8 ± 6.9	47.7 ± 6.6	46.1 ± 6.7	43.8 ± 6.1	50.1 ± 7.9	0.874	0.314	0.233	0.041*	0.281	0.219	0.194	0.029*	0.001*
IPL thickness (μm)	38.9 ± 3.9	38.5 ± 4.1	38.1 ± 4.2	36.9 ± 4.1	35.2 ± 3.8	41.7 ± 3.5	0.649	0.408	0.045*	0.026*	0.005*	0.019*	ʿ0.001*	ʿ0.001*	< 0.001*
Outer Ring (Perifoveal)															
RNFL thickness (μm)	35.9 ± 2.3	35.8 ± 2.6	35.6 ± 2.8	34.4 ± 2.6	32.8 ± 2.1	37.3 ± 2.7	0.961	0.682	0.016*	< 0.001*	0.030*	0.034*	0.013*	ʿ0.001*	< 0.001*
GCL thickness (μm)	37.1 ± 2.6	37.6 ± 1.6	37.03±2.5	36.6 ± 2.9	35.03±2.7	38.4 ± 1.9	0.432	0.830	0.423	< 0.001*	0.039*	0.231	0.014*	0.005*	< 0.001*
IPL thickness (μm)	29.9 ± 2.5	29.7 ± 2.5	29.3 ± 2.4	28.1 ± 3.3	27.03±3.5	31.5 ± 2.1	0.772	0.309	0.011*	0.006*	0.008*	0.003*	ʿ0.001*	ʿ0.001*	< 0.001*

SD: Standard deviation; RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer.

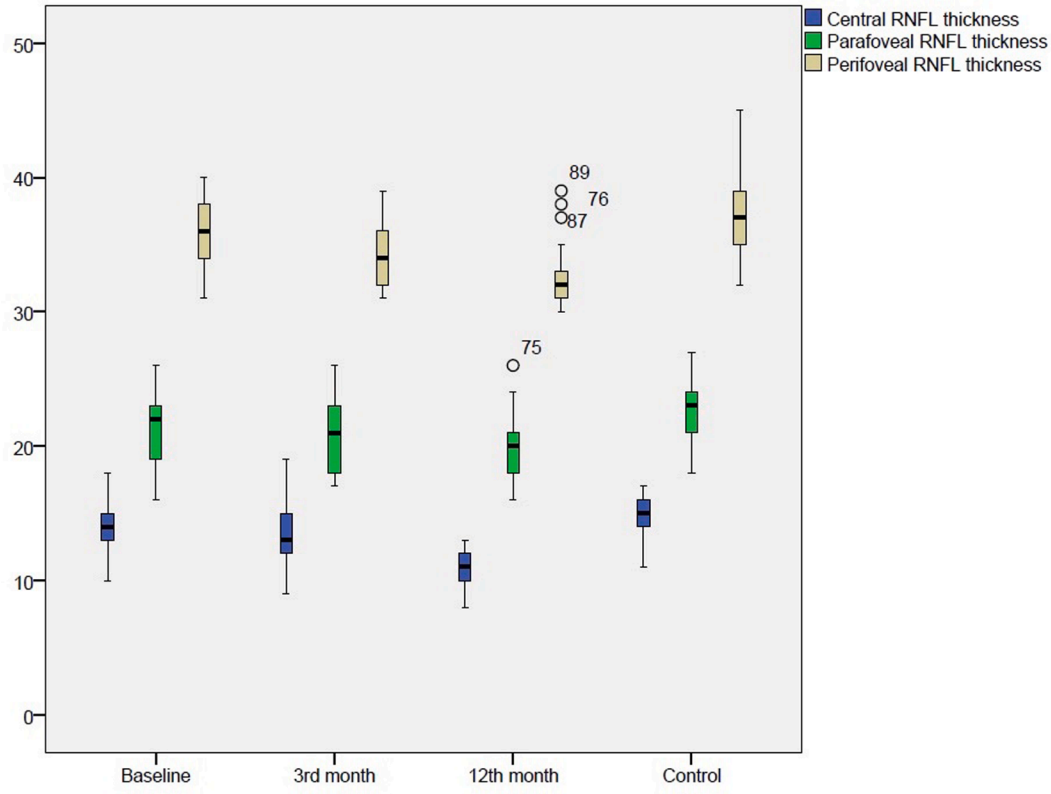

Fig. 1. The distribution of mean central, parafoveal, and perifoveal RNFL thickness.

= 0.029, respectively). The mean IPL thickness was statistically significantly different when compared to both the baseline and the controls (p = 0.045 and p ˂ 0.001, respectively).

At 12 months, the mean RNFL thickness was statistically significantly different when compared to the controls ($p < 0.001$). There was a statistically significant difference for GCL and IPL at 12 months when

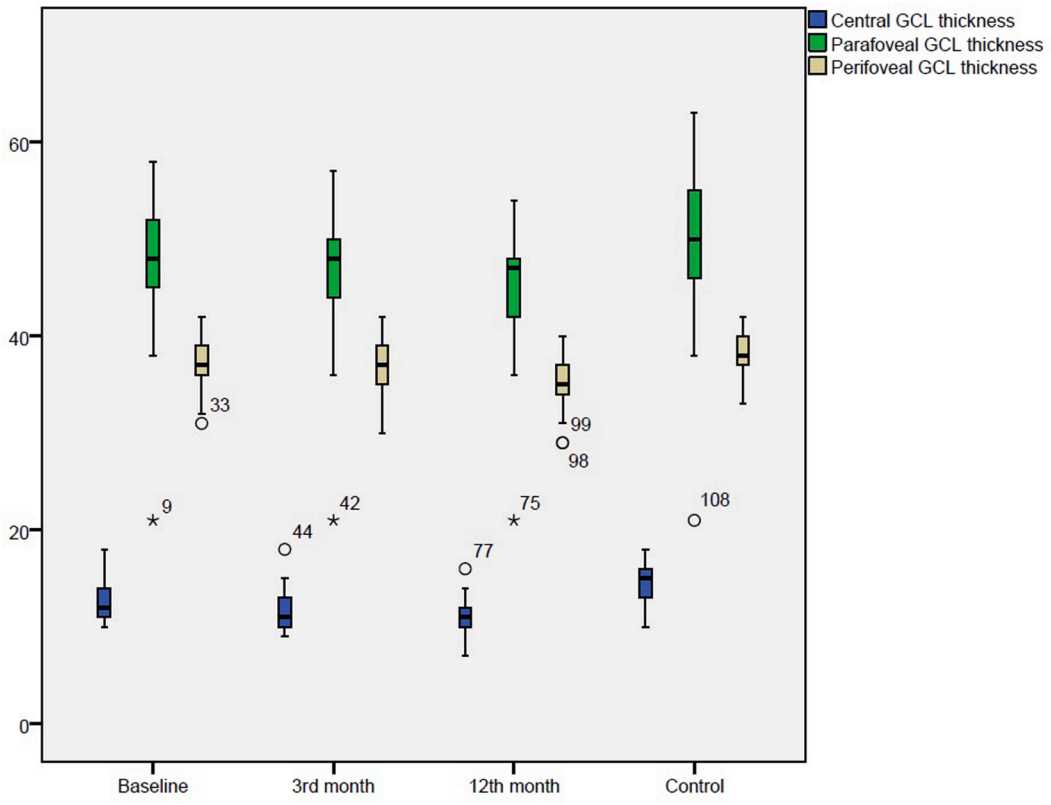

Fig. 2. The distribution of mean central, parafoveal, and perifoveal GCL thickness.

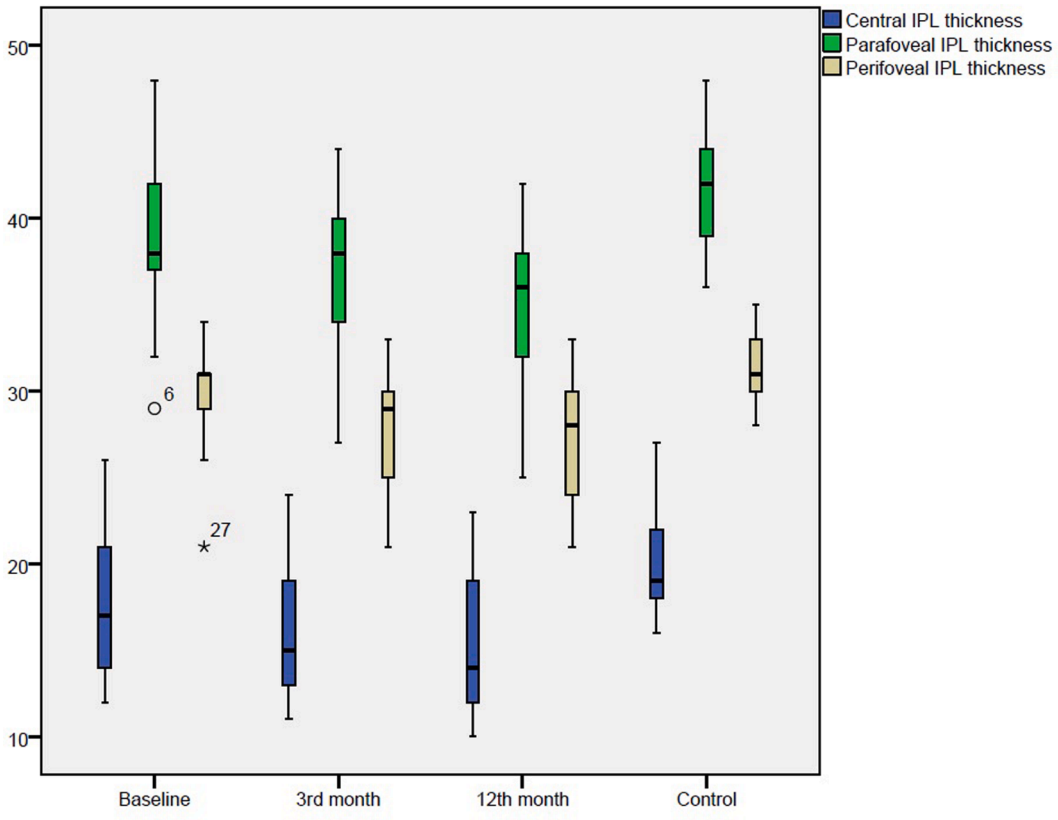

Fig. 3. The distribution of mean central, parafoveal, and perifoveal IPL thickness.

compared to both the baseline and the controls ($p = 0.041$, $p = 0.026$ and $p = 0.001$, $p < 0.001$, respectively).

In the perifoveal region of the ETDRS grid, there was a statistically significant difference between the patient group and the control group for the RNFL, GCL, and IPL thicknesses at the baseline ($p = 0.030$, $p = 0.039$, and $p = 0.008$, respectively).

After the first injection, the mean RNFL and GCL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.961$ and $p = 0.432$, respectively). The mean RNFL and IPL thicknesses were statistically significantly different when compared to the controls ($p = 0.034$ and $p = 0.003$, respectively). The mean IPL thickness was not statistically significantly different when compared with the baseline ($p = 0.772$), and the mean GCL thickness was not statistically significantly different when compared with the controls ($p = 0.231$).

After the second injection, the mean RNFL and GCL thicknesses were not statistically significantly different when compared with the baseline ($p = 0.682$ and $p = 0.830$, respectively), but they were statistically significantly different when compared to the controls ($p = 0.013$ and $p = 0.014$, respectively). The mean IPL thickness was statistically significantly different when compared to the baseline ($p = 0.309$), yet it was not statistically significantly different when compared to the baseline ($p < 0.001$).

After the third injection, the mean RNFL thickness was statistically significantly different when compared to the baseline ($p = 0.016$). The mean GCL thickness did not show a statistically significant difference when compared with the baseline ($p = 0.423$). The mean RNFL and GCL thicknesses were statistically significantly different when compared to the controls ($p < 0.001$ and $p = 0.005$, respectively). The mean IPL thickness was statistically significantly different when compared to both the baseline and the controls ($p = 0.011$ and $p < 0.001$, respectively).

The mean RNFL, GCL, and IPL thicknesses were found to be statistically significantly different at 12 months when compared to both the baseline and the control eyes ($p < 0.001$, $p < 0.001$; $p < 0.001$, $p < 0.001$; and $p = 0.006$, $p < 0.001$, respectively).

The mean RNFL, GCL, and IPL thicknesses in the central, parafoveal, and perifoveal rings are listed in Table 1.

The distribution of the mean central, parafoveal, and perifoveal RNFL, GCL, and IPL thicknesses are shown in Figs. 1, 2, and 3.

4. Discussion

The results of this study showed significantly decreased macular GCC thickness in patients with nAMD after intravitreal aflibercept injection, particularly at 12 months. To the best of our knowledge, no prior study has evaluated the effects of intravitreal aflibercept injection on RNFL, GCL, and IPL thicknesses in patients with nAMD.

As the treatment of nAMD with intravitreal anti-VEGF injection is known to have an excellent outcome, most patients will need long-term repeated injections. However, despite the efficacy of anti-VEGF agents, ocular side effects are still unclear.

Besides its role in the development of nAMD, VEGF-A has been shown to have a neuroprotective function in both the GCL and the RNFL [11]. In a review of studies evaluating the effects of anti-VEGFs on retinal ganglion cells, a laboratory study showed that VEGF protects retinal ganglion cells from oxidative stress, and this protective effect is eliminated by treatment with bevacizumab [12]. A similar study reported that the survival of retinal ganglion cells decreased with increasing concentrations of bevacizumab administration [13].

Previous clinical studies on retinal changes following intravitreal injection of anti-VEGF agents mostly reported changes in RNFL assessed via OCT. Entezari et al. found that RNFL thickness was significantly decreased at 12 weeks after treatment with 2 intravitreal bevacizumab injections in patients with nAMD, while there was no significant difference at 24 weeks when compared with the baseline [14]. Conversely, in another study, it was found that long-term treatment with anti-VEGF

agents caused no significant change in RNFL thickness in patients with AMD [15]. Beck et al. reported that retinal GCL thickness was significantly decreased, without any significant change in RNFL thickness after an average of 31.5 anti-VEGF injections [16].

According to the results of our study, during the 12-month follow-up period, the mean GCL, RNFL, and IPL thicknesses were significantly decreased in the central, parafoveal, and perifoveal macular areas. It has been reported that decreased GCC thickness after intravitreal anti-VEGF may result from progressive arteriolar vasoconstriction, including consequent retinal ischemia associated with glutamate release, which may damage retinal ganglion cells, particularly those that are sensitive to the substance [17,18]. In the patient group, IOP elevation was not observed during the follow-up period. Therefore, decreased thickness of the GCL-IPL and RNFL could not be associated with IOP changes. As shown in previous studies, RNFL thickness changes could be observed in eyes with AMD and were mainly linked to either the transient increase in intraocular volume produced by the injection or through a direct toxic effect of the anti-VEGFs on the RNFL.

Lee and Yu found decreased GC-IPL and RNFL thicknesses in eyes with dry AMD when compared to the controls [19]. Zucchiatti et al. reported similar results in eyes with nAMD [20]. However, Lee et al. showed that the number of injections, type of anti-VEGF agent, and duration of treatment were not associated with the amount of GCL-IPL thinning [21]. We cannot fully exclude the possibility that the decreased GCC thickness may be attributable to the natural course of the disease. Therefore, GCC thickness changes during long-term anti-VEGF treatment, given the relative stability of the disease, suggest that our findings in the patient group may be associated with intravitreal aflibercept treatment.

This study has some limitations, such as the small number of participants and its retrospective design. Additionally, automated segmentation may show artifacts in the inner retinal layers, and there may be segmentation errors causing overestimation or underestimation of the GCC thickness.

5. Conclusion

Although intravitreal anti-VEGF treatment is relatively safe, careful follow-up is suggested post-injections to observe the possibility of GCC changes in patients with nAMD. Further longitudinal studies with a larger sample size are needed to clarify the issue.

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References

[1] J.B. Christoforidis, N. Tecce, R. Dell'Omo, R. Mastropasqua, M. Verolino, C. Costagliola, Age related macular degeneration and visual disability, Curr. Drug Targets 12 (2) (2011) 221–233, https://doi.org/10.2174/138945011794182755.

[2] V.S. Jeganathan, N. Verma, Safety and efficacy of intravitreal anti-VEGF injections for age-related macular degeneration, Curr. Opin. Ophthalmol. 20 (3) (2009) 223–225, https://doi.org/10.1097/ICU.0b013e328329b656.

[3] P.J. Rosenfeld, D.M. Brown, J.S. Heier, D.S. Boyer, P.K. Kaiser, C.Y. Chung, R. Y. Kim, MARINA Study Group, Ranibizumab for neovascular age-related macular degeneration, N. Engl. J. Med. 355 (14) (2006) 1419–1431, https://doi.org/10.1056/NEJMoa054481.

[4] R.L. Avery, D.J. Pieramici, M.D. Rabena, A.A. Castellarin, M.A. Nasir, M.J. Giust, Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration, Ophthalmology 113 (3) (2006) 363–372, https://doi.org/10.1016/j.ophtha.2005.11.019, e5.

[5] C.A.T.T. Research Group, D.F. Martin, M.G. Maguire, G.S. Ying, J.E. Grunwald, S. L. Fine, G.J Jaffe, Ranibizumab and bevacizumab for neovascular age-related macular degeneration, N. Engl. J. Med. 364 (20) (2011) 1897–1908, https://doi.org/10.1056/NEJMoa1102673.

[6] D.J. Browning, P.K. Kaiser, P.J. Rosenfeld, M.W. Stewart, Aflibercept for age-related macular degeneration: a game-changer or quiet addition? Am. J. Ophthalmol. 154 (2) (2012) 222–226, https://doi.org/10.1016/j.ajo.2012.04.020.

[7] H.J. Cho, S.G. Yoo, H.S. Kim, J.H. Kim, C.G. Kim, T.G. Lee, J.W. Kim, Risk factors for geographic atrophy after intravitreal ranibizumab injections for retinal angiomatous proliferation, Am. J. Ophthalmol. 159 (2) (2015) 285–292, https://doi.org/10.1016/j.ajo.2014.10.035, e1.

[8] M.S. Zinkernagel, P. Schorno, A. Ebneter, S. Wolf, Scleral thinning after repeated intravitreal injections of antivascular endothelial growth factor agents in the same quadrant, Invest Ophthalmol. Vis. Sci. 56 (3) (2015) 1894–1900, https://doi.org/10.1167/iovs.14-16204.

[9] K. Nishijima, Y.S. Ng, L. Zhong, J. Bradley, W. Schubert, N. Jo, J. Akita, S. Samuelsson, G.S. Robinson, A.P. Adamis, 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. 171 (1) (2007) 53–67, https://doi.org/10.2353/ajpath.2007.061237.

[10] C.K. Cheng, P.H. Peng, L.T. Tien, Y.J. Cai, C.F. Chen, Y.J. Lee, Bevacizumab is not toxic to retinal ganglion cells after repeated intravitreal injection, Retina 29 (3) (2009) 306–312, https://doi.org/10.1097/IAE.0b013e3181909404.

[11] R.H. Foxton, A. Finkelstein, S. Vijay, A. Dahlmann-Noor, P.T. Khaw, J.E. Morgan, D.T. Shima, Y.S. Ng, VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma, Am. J. Pathol. 182 (4) (2013) 1379–1390, https://doi.org/10.1016/j.ajpath.2012.12.032.

[12] V.S. Brar, R.K. Sharma, R.K. Murthy, K.V. Chalam, Bevacizumab neutralizes the protective effect of vascular endothelial growth factor on retinal ganglion cells, Mol. Vis. 16 (2010) 1848–1853.

[13] J.M. Lee, H.W. Bae, S.Y. Lee, G.J. Seong, C.Y. Kim, Effect of anti-vascular endothelial growth factor antibody on the survival of cultured retinal ganglion cells, Korean J. Ophthalmol. 31 (2017) 360–365, https://doi.org/10.3341/kjo.2017.0054.

[14] M. Entezari, A. Ramezani, M. Yaseri, Changes in retinal nerve fiber layer thickness after two intravitreal bevacizumab injections for wet type age-related macular degeneration, J. Ophthalmic Vis. Res. 9 (2014) 449–452, https://doi.org/10.4103/2008-322X.150815.

[15] M.B. Horsley, N. Mandava, M.A. Maycotte, M.Y. Kahook, Retinal nerve fiber layer thickness in patients receiving chronic anti-vascular endothelial growth factor therapy, Am. J. Ophthalmol. 150 (2010) 558–561, https://doi.org/10.1016/j.ajo.2010.04.029.

[16] M. Beck, M.R. Munk, A. Ebneter, S. Wolf, M.S. Zinkernagel, Retinal ganglion cell layer change in patients treated with anti-vascular endothelial growth factor for neovascular age-related macular degeneration, Am. J. Ophthalmol. 167 (2016) 10–17, https://doi.org/10.1016/j.ajo.2016.04.003.

[17] K.S. Kim, H.R. Chang, S. Song, Ischaemic change after intravitreal bevacizumab (Avastin) injection for macular oedema secondary to non-ischaemic central retinal vein occlusion, Acta Ophthalmol. 86 (8) (2008) 925–927, https://doi.org/10.1111/j.1755-3768.2008.01175.x.

[18] R. Siliprandi, R. Canella, G. Carmignoto, N. Schiavo, A. Zanellato, R. Zanoni, G. Vantini, N-methyl-D-aspartate-induced neurotoxicity in the adult rat retina, Vis. Neurosci. 8 (6) (1992) 567–573, https://doi.org/10.1017/s0952523800005666.

[19] E.K. Lee, H.G. Yu, Ganglion cell–inner plexiform layer and peripapillary retinal nerve fiber layer thicknesses in age-related macular degeneration, Invest. Ophthalmol. Vis. Sci. 56 (2015) 3976–3983, https://doi.org/10.1167/iovs.15-17013.

[20] I. Zucchiatti, M.B. Parodi, L. Pierro, M.V. Cicinelli, M. Gagliardi, N. Castellino, F. Bandello, Macular ganglion cell complex and retinal nerve fiber layer comparison in different stages of age-related macular degeneration, Am. J. Ophthalmol. 160 (2015) 602–607, https://doi.org/10.1016/j.ajo.2015.05.030.

[21] W.J. Lee, Y.K. Kim, Y.W. Kim, J.W. Jeong, S.H. Kim, J.W. Hea, H.G. Yu, K.H. Park, Rate of macular ganglion cell-inner plexiform layer thinning in glaucomatous eyes with vascular endothelial growth factor inhibition, J. Glaucoma 26 (2017) 980–986, https://doi.org/10.1097/IJG.0000000000000776.