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ORIGINAL PAPER

Long-term changes in retinal layers in patients undergoing intravitreal ranibizumab for neovascular age-related macular degeneration

Retinal layers after anti-VEGF therapy

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Abstract

Purpose To analyze long-term changes in individual retinal layers (RLs) after intravitreal injections of ranibizumab (IVRs) in patients with neovascular age-related macular degeneration (n-AMD).

Methods The patients were treated with 0.5-mg IVRs based on an as-needed protocol after the first three monthly doses over a 12-month follow-up period. Patients underwent optical coherence tomography and best-corrected visual acuity (BCVA) evaluation at each visit. The ETDRS grid with central subfield (R1) (r 0.5 mm) and the inner ring (R2) (r 0.5–1.5 mm) was used for calculation of the mean thickness of each RL. Changes in the thickness of segmented RLs within the R1 and R2 of ETDRS circles at months-3, -6, and -12 were compared to baseline.

Results The mean age was 72 ± 7.4 years. The mean number of injections was 9.08 (range 6–11). Mean BCVA improved from 49.7 ± 22.1 to 60.1 ± 19.8 letters. Central macular thickness decreased from 390.25 ± 149.6 to 312.74 ± 118.4 µm. Thicknesses of GCL (from 23.93 ± 13.73 to 19.50 ± 9.50 µm in R1; p 0.001, and from 44.5 ± 12.6 to 39.6 ± 10.6 µm in R2; p 0.005), IPL (from 28.90 ± 14.36 to 22.35 ± 6.23 µm in R1; p 0.001, and from 39.34 ± 8.53 to 35.58 ± 7.93 µm in R2; p 0.004), and total inner RL (ILM to ELM) (from 222.93 ± 93.09 to 180 ± 53 µm in R1; p 0.001, and from 255.06 ± 42.74 to 240.25 ± 40.37 µm in R2;

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p 0.003) in the central and parafoveal rings decreased statistically at month-12. Decrease in INL was limited to month-6 (from 34.80 ± 15.33 to 27.60 ± 12.59 μm in R1; p 0.001), while decreases in total outer RLs (ELM to RPE) (from 128.32 ± 26.92 to 115.54 ± 43.98 μm in R1; p 0.001, and 103.81 ± 16.73 to 96.38 ± 16.22 μm in R2; p 0.014) and RPE (from 39.12 ± 22.33 to 29.70 ± 22.05 μm in R1; p 0.001, and from 31.27 ± 13.11 to 24.40 ± 9.99 μm in R2; p 0.001) were limited to month-3.

Conclusions Significant changes were observed in the thickness of the inner RLs after 1-year treatment with IVRs for n-AMD. A significant decrease in RPE thickness confined to the first months disappeared at month-12.

Keywords Neovascular age-related macular degeneration · Macular edema · Retinal layers · Retinal segmentation

Introduction

Neovascular age-related macular degeneration (n-AMD) is the most frequent cause of central vision loss in the elderly population [1]. The role of vascular endothelial growth factor (VEGF) in the pathogenesis of AMD has been shown [2]. Ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA, USA) is a monoclonal antibody fragment exerting pan-VEGF inhibition with increased VEGF affinity [3]. The beneficial effects of ranibizumab treatment in patients with n-AMD have been reported in multicenter, randomized clinical trials, and real-world studies [2, 4]. Vascular endothelial growth factor and its receptors undergo constant release in normal eyes at a basal level and are shown to be important in neurogenesis, neuron migration and protection, and neural dissociation [2, 3, 5, 6]. When these effects of VEGF are taken into consideration, it might be hypothesized that anti-VEGF agents, which can penetrate all retinal layers when administered through an intravitreal route, may cause a loss of retinal neuron cells over long-term use [7]. Damage to ganglion cells and retinal nerve fibers is also possible due to a transient or permanent increase of intraocular pressure (IOP) with repeated intravitreal injections [8, 9].

While there are findings of potential side effects of VEGF inhibition in some preclinical studies, no side effects on retinal cells have been clinically found [10–14]. Intravitreal ranibizumab injections were clinically accepted to have no functional ocular side effects [13, 14]. However, whether any retinal layer is influenced structurally from the VEGF inhibition or natural progression of n-AMD without any significant function loss is unknown. Such an effect may be due to the secondary effects of the edema leaking from neovascularization or degenerative processes as well as the effects of anti-VEGF agents. Retinal pigment epithelium (RPE) atrophy, which is an ongoing topic of discussion regarding whether it is related to anti-VEGF treatment or the natural course of AMD, was reported in clinical trials [15]. However, the structural effects of ranibizumab on individual retinal layers in the macular region have not been studied. Thickness in retinal layers can be measured with new segmentation analysis software [16, 17]. We designed our study to analyze each retinal layer thickness during ranibizumab treatment for over 12 months. According to best of our knowledge, our study is the first study investigating changes in the thickness of each retinal layer after long-term anti-VEGF therapy.

Methods

Thirty-seven consecutive patients with n-AMD were enrolled in this prospective study between January 2015 and March 2016. All patients were naïve to the anti-VEGF treatment. Afyonkarahisar Clinical Trials Ethics Committee approved our study. Each patient was informed about the procedures and tests. Written informed consent was taken from each patient. All cases underwent routine ophthalmologic examination before enrollment. Visual acuity was measured with an ETDRS chart and recorded as the number of letters. Care was taken to make the patients read a maximum number of letters in each examination. Following the slit-lamp examination, IOP was measured with applanation tonometry. The baseline examination included binocular stereoscopic fundus evaluation, optical coherence tomography (OCT), color fundus images, and fundus fluorescein angiography (FFA) (Zeiss Visucam 500, Carl Zeiss Meditec AG, Oberkochen, Germany). The patients were monitored with BCVA and OCT in monthly visits.

Patients were administered with monthly intravitreal ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA, USA) injections (IVRs) consecutively in the first 3 months and later in 1-month intervals until stabilization was observed. Additional doses were administered as needed if any activation was observed during monthly follow-up visits. Activation criteria were accepted to be a new fluid pattern in OCT scanning (intraretinal or subretinal), a loss of five letters or more in visual acuity, or the presence of recent hemorrhage in fundus examination or photos.

SD-OCT scan acquisition of foveal retinal layers

All scans were obtained by a spectral-domain OCT device (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany) in a dark room without pupil dilatation. Complying with the instructions for the use of the device, mean keratometric values of patients were entered into Spectralis software before scanning. Two consecutive 20° × 20° volume scans were obtained. The volume scan was comprised of 49 parallel B-scans separated by 120 μm, whereby each B-scan was an average of 9 frames ART (automatic real-time: 9), each consisting of 512 A-scans. Scan quality was accepted above 25 decibels (dB) in accordance with the manufacturer guidelines. Center fixation point thickness (CFPT), central subfield thickness (CSFT), central macular volume (CMV), and total macular volume (TMV) measurements were recorded. The Heidelberg Eye Explorer software (HEYEX version 1.9.10.0; Spectralis Viewer Module 6.3.x.x; Spectralis Acquisition Module 6.3.x.x; Heidelberg Engineering GmbH, Heidelberg, Germany) was used for automated retinal layer segmentation (Supplemental Figure 1). When an error was detected in transitions between segments in automatic segmentation, the current manual adjustment mode was used to adjust segmentation lines. Segmentation measures were re-calculated and recorded. Provided standard ETDRS grid with central subfield (r 0.5 mm), the inner ring (r 0.5–1.5 mm), and outer ring (r 1.5–3 mm) was used for calculation of the mean thickness of each retinal layer within the corresponding areas. Thicknesses of the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and RPE were recorded. Additionally, inner retinal layers (IRL) measuring the thickness between ILM and ELM (RNFL + GCL + IPL + INL + OPL + ONL); and outer retinal layer (ORL) measuring the thickness between ELM and BM (Photoreceptor + RPE complex) were also recorded. Although the inner retina has been defined as the summation of RNFL, GCL, IPL, and INL based on the metabolic supply, IRL has been given as summation of RNFL + GCL + IPL + INL + OPL + ONL depicted by the software. Superior, nasal, inferior, and temporal sectors of the parafoveal region, which is the second circle in the ETDRS grid, were also measured. Retinal segmentation analysis was performed at baseline, month-3, month-6, and month-12.

Inclusion criteria were as follows: recently diagnosed with n-AMD and a history of symptoms for no more than 6 months; being treatment-naïve for n-AMD; and a minimum visual acuity of 20/200. Patients with the following conditions were not included in the study: disciform scar in the macula center; lesions of complex configuration without a reliable retinal segmentation; opacity of the cornea and/or lens that might hinder OCT scanning; retinal vascular diseases such as vein occlusion, diabetic retinopathy and hypertensive retinopathy; anterior or posterior segment operation done previously or during the study period; glaucoma; or inflammatory eye pathology. The followings were considered as additional exclusion criteria: not showing up for examination visits, requesting to discontinue treatment, endophthalmitis, retinal detachment, any complications such as intravitreal bleeding related to intravitreal injections, or a systemic complication related to a treatment agent.

Statistical analysis

SPSS (Statistical Package for Social Science, 17.0 Worldwide Headquarters SPSS Inc.) was used for statistical evaluation. After comparison of BCVA, IOP, macular thickness, and retinal segmentation with baseline values and distribution analyses, we then conducted general linear models, paired sample t tests, or Wilcoxon tests. Because there are repetitive measures, Bonferroni correction was used for the general linear models. p Values below 0.016 were considered as significant.

Results

Two patients were excluded from the study because they missed the follow-up visits. Data from two patients were excluded from the analyses because their OCT scans were unreliable due to segmentations artifacts during the follow-up visits. Monthly examinations were conducted in 33 patients. Baseline characteristics are given in Table 1. None of the patients had any complication such as a retinal tear, retinal detachment, vitreous hemorrhage, or endophthalmitis.

Visual acuity and IOP

Baseline mean BCVA was 49.7 ± 22.1 letters and increased to 57.8 ± 22.5 letters in month-3, 59.1 ± 19.9 letters in month-6, and 60.1 ± 19.8 letters in month-12. The number of patients with 20/40 or better BCVA before the treatment, at months-3, -6, and -12 was 9 (27.3%), 14 (42.2%), 15 (45.4%), and 17 (51.5%), respectively. BCVA increased at least three lines in seven patients at month-12. Statistically significant improvements in BCVA were observed starting from month-3. In our study, none of the cases experienced ≥ three levels of decrease in visual acuity at month-12. Increase in mean visual acuity was 10.4 letters. No significant change was seen in IOP during follow-up (Snellen BCVAs are provided in Table 2).

Table 1 Patient demographics

Gender	18 M 15 F
Mean age (years)	72 ± 7.4 (56–83)
Laterality	
Right eye	15
Left eye	18
Number of intravitreal injections	9.08 ± 1.63 (6–11)
Follow-up	12-month
Lens status	
Pseudophakic	8
Phakic	25
Angiographic type of CNV	
Occult (77.4%)	25
Predominantly classic (22.6%)	8
Lesion size	
< 1 disk diameter (DD)	5
1 DD	9
Between 1 DD and 2 DD	15
> 2 DD	4
Fluid patterns of lesion	
Subretinal fluid	19
Cystoid fluid pattern	6
Subretinal and cystoid fluid pattern	8
Fluid plus RPE detachment (PED)	28

Retinal segmentation analysis

Baseline CFPT, SFMT, SMV, and TMV improved statistically at months-3, -6, and -12 (Table 2). In segmentation analysis, the thickness of RNFL did not show a significant change in any sector during the follow-up. At month-12, the thickness of GCL decreased significantly in the central 1 mm area, in the mean of the 1–3 mm area, and the superior, inferior, temporal quadrants of the 1–3 mm area. The thickness of IPL decreased significantly in the central 1 mm area, in the mean of the 1–3 mm area, and the temporal quadrant of the 1–3 mm area. A statistically significant decrease in the thickness of INL in the central 1 mm area was observed at month-6. In the segmental analysis of OPL and ONL, no statistically significant changes were found in any of the sectoral quadrants during the follow-up. At month-3, the thickness of RPE decreased significantly in the central 1 mm region, in the mean of the 1–3 mm area, and the superior, inferior, and temporal quadrants of the 1–3 mm area. A decrease in RPE was maintained only in the superior quadrant of the 1–3 mm area and in the mean of the 1–3 mm area at month-6. The thickness of IRL decreased significantly in the temporal quadrant of the 1–3 mm area at month-3, in the inferior quadrant of the 1–3 mm area at month-6, and in the mean of the 1–3 mm area and the central 1 mm area at month-12. The thickness of ORL decreased significantly in the central 1 mm area, in the nasal, inferior, and temporal quadrants of the 1–3 mm area at month-3 and in the mean of the 1–3 mm area at month-6. The central 1 mm area foveal and 1–3 mm parafoveal measurements are given in Table 3. Total analyses in all sectors are presented in supplemental Tables 1–5.

Table 2 Functional and anatomical outcomes over 12 months of follow-up after intravitreal ranibizumab injections

	Best-corrected visual acuity		Intraocular pressure (mmHg)	Center point macular thickness (μm)	Central subfield macular thickness (μm)	Central macular volume	Total macular volume
	ETDRS Letters Mean + SD	Snellen Mean (Min–max)					
Baseline	49.7 ± 22.1	20/87 (32–200)	13.70 ± 2.77	406.5 ± 145.9	381.6 ± 110.2	0.290 ± 0.08	8.97 ± 0.95
Month-3	57.8 ± 22.5	20/67 (25–160)	13.29 ± 1.84	331.9 ± 139.7	329.6 ± 79.9	0.254 ± 0.06	8.34 ± 0.95
Month-6	59.1 ± 19.9	20/64 (20–160)	13.87 ± 2.45	322.2 ± 136.4	333.4 ± 99.4	0.256 ± 0.08	8.39 ± 1.08
Month-12	60.1 ± 19.8	20/64 (20–160)	14.77 ± 2.36	294.2 ± 110.3	318.8 ± 77.1	0.254 ± 0.06	8.37 ± 0.87
p 0–3	**< 0.001**		0.259	**0.001**	**0.001**	**0.002**	**< 0.001**
p 0–6	**< 0.001**		0.654	**0.002**	**0.013**	**0.014**	**0.002**
p 0–12	**< 0.001**		0.061	**0.001**	**< 0.001**	**0.002**	**< 0.001**

SD standard deviation, *p 0–3* comparison of baseline and month-3, *p 0–6* comparison of baseline and month-6, *p 0–12* comparison of baseline and month-12

Bold values show statistically significant measurements

Discussion

In our study, we found a decrease in the thickness of GCL, IPL, INL, IRL, and RPE in some quadrants of ETDRS circle. We found insignificant thinning in mean baseline RNFL values of the foveal, parafoveal, and parafoveal sectors in retinal segmentation analysis following 12 months of ranibizumab treatment with a mean of 9.08 injections. Our results seem to support some previous studies. The most assessed retinal layer has previously been the RNFL following long-term anti-VEGF treatment in patients with n-AMD [18–23]. However, most studies included peripapillary RNFL evaluation, while RNFL change in the macula by segmentation analysis has not been studied as much [18–23]. Previous studies have reported conflicting results on the peripapillary RNFL changes after anti-VEGF treatment. While some reported no difference in the thickness of the RNFL, statistically significant thinning in the peripapillary RNFL after ranibizumab therapy has also been reported [18–22]. Controlled clinical trials have provided possible evidence linking repeated anti-VEGF injection to an increased risk for RNFL loss [23]. These studies have not been consistent with one another, possibly due to differences in study designs [23]. RNFL loss reported in previous studies may suggest neutralization of neuroprotective effects of VEGF or temporary IOP fluctuations seen after each intravitreal injection [9, 18, 21, 22]. The lack of significant decrease in macular RNFL thickness observed in our study might be due to underestimation of baseline RNFL thickness as a result of microvascular changes in outer retinal layers and suppression of inner retinal layers by the edema [20]. Thinning of RNFL in the natural course of AMD may occur due to the cessation of development of collateral innervation between macular neurons or development of irreversible neuronal interconnections secondary to choroidal neovascularization [24]. Although an age-related decrease in RNFL thickness has been demonstrated in healthy subjects [25], we did not find a significant reduction of RNFL thickness over 12 months possibly due to reasons aforementioned.

We found a significant decrease in GCL by segmentation analysis of Spectralis OCT. Significant damage was reported in the GCL following intravitreal VEGF blockade in animal models [5]. VEGF-A may act as a survival factor for RGCs. Apoptosis of RGCs has been demonstrated to increase in diabetic rats treated with an anti-VEGF agent. [26]. Furthermore, VEGF inhibition has been shown to block the protective effect of VEGF on RGCs [27]. So, repeated

Table 3 Changes in thickness of each segmented retinal layer at, months-3, -6, and -12 during time course under intravitreal ranibizumab therapy in patients with n-AMD

Time	Retinal nerve fiber layer thickness (μm)		Ganglion cell layer thickness (μm)		Inner plexiform layer thickness (μm)		Inner nuclear layer thickness (μm)	
	Foveal (1 mm)	Parafoveal (1–3 mm)	Foveal (1 mm)	Parafoveal (1–3 mm)	Foveal (1 mm)	Parafoveal (1–3 mm)	Foveal (1 mm)	Parafoveal (1–3 mm)
Baseline	25.0 ± 18.3	29.3 ± 12.1	23.9 ± 13.7	44.5 ± 12.6	28.9 ± 14.3	39.3 ± 8.5	34.8 ± 15.3	46.1 ± 8.2
Month-3	21.6 ± 15.9	26.1 ± 8.9	22.2 ± 12.1	42.1 ± 12.7	28.1 ± 16.5	37.8 ± 7.9	30.6 ± 13.7	43.7 ± 6.8
Month-6	25.9 ± 23.1	27.3 ± 11.7	21.3 ± 10.1	43.8 ± 12.0	29.6 ± 17.1	38.6 ± 8.5	28.6 ± 12.5	43.8 ± 7.5
Month-12	23.0 ± 17.5	26.9 ± 10.1	19.5 ± 9.5	39.6 ± 10.6	22.4 ± 6.2	35.5 ± 7.9	28.4 ± 12.6	42,9 ± 5.4
p	0.363*	0.432*	**0.001***	**0.005***	**0.001***	**0.004***	**0.013****	0.191*

Time	Outer plexiform layer thickness (μm)		Outer nuclear layer thickness (μm)		Retinal pigment epithelium thickness (μm)	
	Foveal (1 mm)	Parafoveal (1–3 mm)	Foveal (1 mm)	Parafoveal (1–3 mm)	Foveal (1 mm)	Parafoveal (1–3 mm)
Baseline	30.6 ± 10.8	37.6 ± 7.5	74.3 ± 21.8	64.5 ± 16.4	39.1 ± 22.3	31.3 ± 13.1
Month-3	30.9 ± 1.3	34.7 ± 5.8	75.0 ± 18.4	61.7 ± 14.3	29.7 ± 22.1	24.4 ± 9.9
Month-6	29.9 ± 13.3	35.5 ± 4.8	75.3 ± 16.9	63.6 ± 17.8	31.9 ± 21.9	26.4 ± 13.2
Month-12	29.5 ± 12.4	34.1 ± 4.8	75.3 ± 25.4	63.9 ± 14.2	34.6 ± 19.9	27.5 ± 12.9
p	0.510*	0.093*	0.880*	0.545*	**< 0.001*****	**< 0.001***** **0.049****

Bold values show statistically significant measurements

*Baseline–month-12 *p* value

**Baseline–month-6 *p* value

***Baseline–month-3 *p* value

exposure to anti-VEGF agents may impair RGC homeostasis [28]. However, there is ongoing controversy regarding the changes of the GCL after anti-VEGF therapy [21–30]. Thinning of the GCL has been reported in patients with AMD [21–24]. One study observed that patients with n-AMD had thinner ganglion cell complex compared to control and dry-AMD [29]. However, there are a limited number of studies analyzing changes in GCL thickness in patients with n-AMD following anti-VEGF treatment [28, 30]. Because the macular region contains approximately 50% of all RGCs, it is essential to conduct a functional or a structural assessment of the macular layers to evaluate early GC loss or changes related to the treatment. A recent study showed a significant decrease in the RGCL in eyes with n-AMD compared to the fellow untreated eyes after anti-VEGF therapy with a mean number of 31.5 injections and a mean follow-up period of 45.3 months [28]. This study measured the RNFL and RGCL in the macular region in a way similar to our technique but evaluated the outer ring of the ETDRS grid rather than the central and inner rings as in our study. In contrast to the thinning of RGCL, no significant effects were observed for RNFL. Consistent with that study, we also found significant decreases in the mean GCL thickness in most of the sectoral areas at 12 months. There are several hypotheses for GC loss in n-AMD. One possibility is that disruption of the interaction between glial cells and ganglion cells might cause GC loss in n-AMD [31]. Alternatively, axonal transport blockades related to stress developed in the retina due to neovascular complex might cause degeneration in GC [32]. Another possibility is that constant fluid flow

to the inner retinal layer and mechanical suppression of the outer retinal layer can cause a decrease of thickness in the GCL [32, 33]. Decreases in the GCL in advanced AMD might result from thinning of the inner retinal layers secondary to apoptosis as a result of transneuronal degeneration and photoreceptor loss. Ranibizumab treatment or the natural course of the disease might also affect GCL thickness. Additional studies are needed to determine whether the GCL is affected by repeated anti-VEGF injections or AMD itself. We confer that it is challenging to separate the effects of progressive n-AMD and cumulative anti-VEGF treatment as a reason for observed changes in GCL at month-12. The effects of edema resolution should not be overlooked; however, CMT decreased significantly in the early term, but GCL thickness decreased at month-12.

When IPL analysis was conducted in our study, thinning of the temporal quadrant and mean IPL was observed in the central 1 mm area and the 1–3 mm area following intravitreal ranibizumab treatment at month-12. No significant change in superior, inferior, and nasal 1–3 mm quadrants may imply the effects of n-AMD rather than anti-VEGF treatment. If anti-VEGF therapy would be a reason, it should contain all quadrants. The effect of edema resolution might be appeared at month-12 due to complete drying of the lesion. However, we have no definite conclusion.

We are unaware of any previous study that has analyzed INL change following anti-VEGF treatment in n-AMD. In our study, a statistically significant decrease was observed in the central 1 mm region of the INL during month-6. IRL analysis showed statistically significant thinning of the foveal 0–3 mm area at month-12. Thinning of the IRL may occur as a result of individual decreases, especially in the GCL and IPL. Both the resolution of edema in IRLs and the effects of anti-VEGF treatment may be a reason for the thinning of IRL at month-12. While chronic ischemia due to the natural progression of AMD, inflammation, and genetic factors may have a role in the thinning of the post-receptoral retinal layers (OPL, INL, IPL, GCL), changes in the balance of hypoxia due to anti-VEGF agents can also play a role [34]. Our study may be important for its support of such findings in light of the above-mentioned potential reasons. The GCL–IPL complex has been shown to decrease by 0.12 μm with every year of age, and by 1.61 μm per decade of age [35]. We do not think that this very small amount of decrease related to age-related changes played a role in our 12-month study.

INL + OPL complex was found to be significantly low in AMD in a study investigating OPL and INL together in AMD patients [34]. However, the change in this layer was not assessed under anti-VEGF treatment. We found no statistically significant changes in OPL thickness in the foveal and parafoveal regions at months-3, -6, or -12. Only an insignificant decrease in the OPL was observed.

ONL thickness in AMD patients was reported to be similar to the control group [34]. No significant change in the ONL was reported in any of the ETDRS grid circles at the end of the first year with an average of 6 doses of ranibizumab treatment due to diabetic macular edema [36]. In our study, no statistically significant changes were observed in ONL thickness in the foveal and parafoveal regions at months-3, -6, or -12 under intravitreal ranibizumab treatment, which supports results of this study. The ONL layer has mainly photoreceptors and is the most active layer of the retina. However, the photoreceptors have been stated to be relatively resistant to ischemic distress [37]. Furthermore, functional deficits of the photoreceptors can be returned with oxygen and glucose transport [38]. Because our patients started anti-VEGF treatment right after diagnosis of wet AMD, accumulation of amorphous materials decreased, neovascular complex consumption of oxygen was stabilized, and apoptotic destruction of photoreceptors may have been avoided; therefore, the amount of ONL stayed approximately the same as the mean baseline value. With the rapid decrease in exudation related to the neovascular complex after anti-VEGF treatment, the health of the photoreceptors might improve. As a result, the survival of the photoreceptors from apoptosis might be an explanation of the lack of effects on ONL thickness [34].

In our study, a statistically significant thinning in the RPE layer was observed in the central foveal 0–3 mm area at 3 months. Furthermore, the second circle average had a statistically significant decrease at 6 months. Such reductions are in accordance with the literature [15, 28, 39]. In one study, where the change in RPE was assessed with polarization-sensitive OCT, automatic segmentation analysis, and fundus auto fluorescence following ranibizumab in exudative AMD, thinning in RPE was observed in 2 eyes at month-3, in 4 eyes at month-12, and in 3 eyes at

month-24 [39]. Irregular, intermittent RPE atrophy regions develop, and their sizes increase in wet AMD after frequent anti-VEGF therapy. RPE damage may occur at the initial stage of n-AMD development. Such damage results in depolarized material accumulation in RPE, which can be detected by polarization-sensitive OCT scanning [39]. This material in RPE cells has been shown to decrease fastest in the first 3 months after anti-VEGF treatment [39]. Our findings support the previous literature, as the most significant decrease in RPE thickness occurred following the first 3 months after anti-VEGF treatment, and the reduction detected in RPE thickness did not reach statistical significance at 12 months. As a result, alteration in RPE thickness may be observed due to a potential side effect of anti-VEGF treatment or the natural progression of macular degeneration.

In our study, the thickness of the sum of ORLs generally decreased in the foveal 0–3 mm area at month-3. Furthermore, the mean thickness of ORLs in the second circle decreased at month-6. Resolution of the exudation resulting from neovascular complex after anti-VEGF therapy might be a key factor in this thinning. A decrease in RPE thickness might have also contributed to the thickness of total ORLs. No previous studies have evaluated longitudinal changes in IPL, OPL, ONL, RPE, IRL, and ORL layers during ranibizumab or another anti-VEGF therapy for n-AMD. We believe that our prospective study will provide a significant contribution to the literature. However, we should mention as a limitation of our study that it was not a controlled study because it was not designed to test the changes in the fellow eyes as a control group. Although we assumed that the observed changes are mainly due to anti-VEGF treatment, the effects of n-AMD cannot be overlooked. We, therefore, cannot differentiate if the observed long-term changes in the retinal layers are due to n-AMD or the treatment with intravitreal Ranibizumab. As a limitation of the study method, we consider that it is difficult to study the effect of the disease and the treatment separately.

In conclusion, while significant decreases were not observed in RNFL thickness, significant reductions occurred in the GCL, IPL, RPE, total IRL, and total ORL thickness. While a significant decrease was seen in INL thickness in the foveal region, there was a nonsignificant decrease in the parafoveal region. No significant effects were observed in the foveal and parafoveal regions of OPL and ONL thickness.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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