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An insight on the anatomical and functional consequences of aflibercept therapy in age-related macular degeneration

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ABSTRACT

Background: Evaluation of anatomical and functional recovery of the retina after aflibercept therapy in neovascular age-related macular degeneration.
Materials and methods: This prospective study enrolled 33 eyes of 33 naive age-related macular degeneration patients with an average age of 69 (55−82) years. Following a thorough ophthalmological examination, baseline color fundus photography, optical coherence tomography and fluorescein angiography were used to assess the angiographic characteristics and classification of the lesions. Multifocal electroretinography and microperimetry were recorded. In the first three months, all patients received three consecutive intravitreal aflibercept injections on a monthly basis. After the initial three doses, non-responders received additional afibercept injections. The baseline, 3rd and 6th month data were recorded for analysis.
Results: The baseline average best-corrected visual acuity (1.05 log MAR) improved dramatically to 0.9 log MAR in the 3rd and 6th months, respectively. The baseline average central macular thickness of 358.5 ± 232.1 μm decreased significantly to 273.0 ± 109.9 μm and 245.5 ± 109.3 μm in the 3rd and 6th months, respectively. The average thickness of the central 1 mm macular region decreased significantly from 349.5 ± 96.4 μm to the 3rd and 6th month values of 320.6 ± 101.9 and 290.5 ± 86.4 μm, respectively. While the mean retinal sensitivity increased significantly from 4.7 ± 3.0 dB to 6.9 ± 3.4 Db, local deficit decreased from −11.6 ± 4.6 dB to −9.4 ± 4.6 dB. Significant improvements were also observed in all rings of N1 and P1 waves.
Conclusion: Intravitreal aflibercept therapy resulted in significant morphological improvements that were easily identifiable during the 3rd month. Electrophysiological improvements were delayed only to become statistically significant in the 6th month. However, it has been shown that visual acuity and optical coherence tomography parameters alone may be insufficient for both the morphological and functional assessment of the retina.

1. Introduction

Age-related macular degeneration is a common, chronic, and progressive macular degenerative disorder. This disorder is associated with a loss of central vision due to abnormalities in a photoreceptor/retinal pigment epithelium/Bruch's membrane/choroidal complex often resulting in a geographic atrophy and/or neovascularization. It is also a leading cause of an irreversible visual impairment among elderly, affecting 30–50 million people globally [1–3]. Causes of age-related macular degeneration are multifactorial, influenced by age, ethnic background and a combination of environmental and genetic factors. Its prevalence is approximately 10 % for adults aged 65 years and up to 25 % for adults over 75 years of age [4].

There are two clinical forms of age-related macular degeneration, that is, a dry form (atrophic) and a wet form (neovascular or exudative) [5]. Neovascular age-related macular degeneration is characterized by abnormal blood vessels, sometimes accompanied by serous or hemorrhagic detachment of the retinal pigment epithelium, lipid leakage and disciform scar formation. It has been observed in 10%–15% of the age-related macular degeneration patients and is responsible for up to

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90 % of visual loss as a result of neovascularization-related complications [6,7]. Neovascularization is associated with focal and diffuse deposits in Bruch's membrane, relative hypoxia, age-related cleavages in Bruch's membrane and inflammation. In addition to oxidative damage, chronic inflammation and mutations in the complement system associated with apoptosis, vascular endothelial growth factors play crucial roles in the pathogenesis of neovascular age-related macular degeneration [5–8].

The vascular endothelial growth factor family and receptors, in particular, are the main factors responsible for angiogenesis and vascular permeability. Inhibition of the vascular endothelial growth factor-A, which is predominantly responsible for pathogenesis of neovascular membranes, has been successful in neovascular age-related macular degeneration therapy. With an understanding of the role of the vascular endothelial growth factors in this disease pathogenesis, intravitreal anti-vascular endothelial growth factor agents have taken their place as the most important treatment tool, thus reducing a significant proportion of the associated visual loss problems [7].

Microperimetry allows for a direct comparison of retinal pathology with psychophysical measurements and objective assessment of the fixation patterns. Quantification of the macular threshold and retinal fixation properties helps physicians to better grasp the result of their medical therapy [9]. Multifocal electroretinography assess localized pathologies in the outer retinal layers and to record local electrophysiological responses in different regions of the retina [10]. The relationship between neuroretinal functions and both anatomical and visual outcomes in neovascular age-related macular degeneration patients is extremely significant. Identification of electrophysiological changes at cellular level has thus become extremely essential during therapy.

The current study was therefore designed to determine retinal anatomical and functional improvements after intravitreal aflibercept therapy (Eylea®, Bayer, Leverkusen, Germany) in patients with neovascular age-related macular degeneration.

2. Materials and methods

2.1. Study participants

This prospective single-centered study enrolled 33 naïve patients with choroidal neovascularization secondary to neovascular age-related macular degeneration who were followed-up at Afyonkarahisar University of Health Sciences Department of Ophthalmology. Diagnosis was performed by investigating visual symptoms, optical coherence tomography scans, and fluorescein angiographic features of the patients. Following detailed one-to-one prior information about the disease course, tests to be applied and treatment procedures, intravitreal anti-vascular endothelial growth factor therapy was recommended to naïve patients. The study procedure abided by the ethical standards of the Helsinki Declaration and obtained full approval from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee. Patients compliant to the treatment were included in the study and written informed consent was obtained.

2.2. Intravitreal aflibercept therapy

Initially, intravitreal aflibercept was administered monthly for three consecutive months. Additional doses were administered to non-responsive patients after three loading doses and to patients who experienced worsening of the disease during a monthly follow-up. The worsening criteria for the disease were based on the fact that the central macular thickness increased by $\geq$100 micrometers in the optic coherence tomography scans or if new hemorrhage appeared during the fundus examination or in the fundus photography although there were no signs of intraretinal or subretinal fluid pattern.

2.3. Ophthalmologic examination and optical coherence tomography imaging

A comprehensive ophthalmologic examination was performed, including measurements of the best-corrected visual acuity in minimum angle of resolution (logMAR) and Goldmann applanation tonometry. A slit-lamp biomicroscopy of the anterior and posterior segments was carried out before and after full pupil dilation. The lesions' angiographic features and classification were determined simultaneously using color fundus photography (Visucam 5000, Zeiss Inc., Oberkochen, Germany), optical coherence tomography and fluorescein angiography (Heidelberg Engineering, Heidelberg, Germany).

Central macular thickness, fluid patterns of the macular lesion and accompanying vitreoretinal interface disorders were assessed using optical coherence tomography. Immediately after determination of segmentation errors by the device during measurement of the central macular thickness, the errors were corrected manually by placing reference lines on internal limiting membrane and Bruch's membrane, and then thickness between new reference lines was measured and re-measured after using the device's automatic centralization program.

Automatic retinal segmentation analysis of the parameters was determined by manual corrections, and finally repeated segmentation measurements were recorded. This analysis was performed in a central area of 1 mm designated by the Early Treatment Diabetic Retinopathy Study.

Evaluation of the mean values of superior, nasal, inferior and temporal areas of the retina, that is, 1−3 mm inner ring (pericentral area) and 3−6 mm outer ring (peripheral area) were performed. The segmentation measurements were also calculated separately for each of the above areas in terms of segment thickness and average.

2.4. Electrophysiological studies

Microperimetry and multifocal electroretinography procedures were conducted prior to the 1st injection, only to be repeated in the 3rd and 6th months during the follow-up. All microperimetry procedures were performed by the same clinician using the same device. Each patient was pre-tested and provided with five minutes-visual adaptation allowance before microperimetry. During the procedure, a contralateral eye was kept closed. A Goldman III with white stimulus, a 4-2-1 ladder strategy was used. In the meantime, random 68 stimuli (Humphrey 10-2) were applied to the retina areas while covering the central 10° with a grid. Fixation target was set as a 20-diameter red cross. Background brightness of the device was 1.27 cd/m^2 and the highest stimulus intensity was set at 127 cd/m^2. 200 ms was reflected on a white background with stimulus presentation time. Variable stimuli of the Goldman type magnitude were arranged from 0 to 20 dB. The 4-2-1 ladder strategy was then performed and final value was recorded as the final threshold. The device also tested the same brightness levels in all test locations before moving to the next brightness level. Mean retinal sensitivities were compared by calculating the points selected in a polygon averaged automatically by using microperimetry software. Fundus movements were observed during the examination while the patient was looking at the fixation target in order to evaluate fundus fixation. Automatic tracking system calculated horizontal and vertical shifts according to a reference frame and mapped the eye movements during examination.

All multifocal electroretinography procedures were performed by the same technician using the same device, too. Refraction error was corrected at a distance of 33 cm during the procedure. An active electrode known as electroretinography-jet electrode was placed on the cornea after topical anesthesia. Superficial and oily skin layer was cleaned and wiped with alcohol to increase electrical conductivity prior to an installation of the ground and reference electrodes. An earth electrode was placed slightly above the supraorbital margins in the midline of the forehead and the reference electrode was placed one centimeter ahead of the external canthus in the temporal region. The

electrodes were connected through a junction box for instant signal processing. The contralateral eye was closed and the chin was placed in the chinrest. Fixation was followed with an infrared camera during the procedure. The procedure was performed in accordance with the criteria of the International Society for Clinical Electrophysiology. Image pattern of 61 hexagons was adjusted to produce an equal signal on the monitor screen and recordings from the 61 regions of the retina were recorded in approximately five minutes. Screen resolution was set to 1024 × 768. The patients were seated 33 cm from the screen during the exposure and stimulated in an area of ±30° horizontally and ±24° vertically. The stimulus frequency was 17 Hz, the luminance was set to 100 candles/m^2 and the backlighting was set at 30 candles/m^2. Results with the noise level >5 μV were discarded and the tests were continued until an acceptable noise level was achieved. Again, the results associated with attention loss and the number of rejected stimuli >20 % of the total stimuli were discarded.

During a concentric ring analysis the 'first-row Kernel wave' in each ring of N1, P1 and N2 amplitudes and implicit times were calculated. In this analysis: 0−5° area was included within the 1st ring fixation; 5°-10° area within the 2nd ring fixation; 10°-15° area within the 3rd ring fixation; and 15° area within the 4th ring fixation. The mean values of amplitudes and implicit times were recorded for analysis of all rings.

2.5. Statistics

The data were encoded and transferred to the computer program and PASW Statistics 18.0 (IBM Inc., Armonk, New York, USA) was used for statistical evaluation. General linear model was applied in the comparison between the baseline values and the best-corrected visual acuity, intraocular pressure, optic coherence tomography, retinal sensitivity and multifocal electroretinography findings. All parameters were analyzed for normality. Parametric data were evaluated by paired t-test, whereas non-parametric data were evaluated by Wilcoxon Signed Ranks test. P-values of <0.05 were considered statistically significant. Pearson bivariate correlation analysis was used for the parametric data, while Spearman correlation analysis was used for the non-parametric data.

3. Results

All patients were followed-up for at least six months. The mean number of injections was 4.5. There were 15 patients with diabetes mellitus, 20 with hypertension, and ten with both diabetes mellitus and hypertension. Three patients were removed from the study because of an irregular follow-up. Demographic features of the patients have been demonstrated in Table 1.

There was no statistically significant change in intraocular pressure, with baseline and 6th-month values being 13 mmHg and 12.76 mmHg, respectively (p = 0.704). Three patients had the highest intraocular pressure during the 1st month after injection and were treated with appropriate topical anti-glaucoma agents. Cerebrovascular event occurred in one patient during follow-up. No notable ocular complications such as retinal tear, retinal detachment, intravitreal hemorrhage and endophthalmitis were reported.

The mean baseline best-corrected visual acuity (1.05 logMAR) improved significantly to 0.9 logMAR and 0.54 logMAR during the 3rd and 6th months after therapy, respectively (p = 0; and p = 0) (Table 2).

Table 1
Demographic features of the patients (n=33).

Gender (Male/Female)	16/17
Mean age (year)	69 (55−82)
Laterality (right/left)	16/17
Lens condition (Pseudophakic/Phakic)	10/23

Table 2
Changes of the best-corrected visual acuity during therapy (n=33).

Time interval	Best-corrected visual acuity (logMAR)	95 % Confidence interval		P value
		Lower Limit	Higher Limit	
Baseline	1.05±0.53	0.87	1.24	
3rd month	0.9±0.49	0.73	1.08	0.000
6th month	0.54±0.29	0.44	0.65	0.000

3.1. Optical coherence tomography findings

Statistically significant decreases were observed between baseline and the 3rd- and 6th-month central macular thickness and mean thickness of central 1 mm macular area, respectively (p = 0.007; and p = 0) (Tables 3 and 4). Additionally, statistically significant decreases in the: a) inner, outer and central ring thickness of retinal nerve fiber layer, internal nuclear layer, outer plexiform layer, and internal retinal layers, respectively (p = 0; p = 0; and p = 0); b) inner and outer ring thickness parameters of ganglion cell layer, respectively (p = 0.038; and p = 0.002); c) inner ring thickness parameters of internal plexiform layer (p = 0), retinal pigment epithelium (p = 0.366) and internal retinal layers; d) outer ring thickness parameters of outer nuclear layer (p = 0.079); and e) inner and central ring thickness parameters of outer retinal layer, respectively (p = 0; and p = 0.028) were observed (Table 5).

3.2. Microperimetry data analysis

There were statistically significant changes in fixation stability analysis and fixation localization, as well as retinal sensitivity and local deficit analysis (p < 0.001) (Tables 6–9).

3.3. Multifocal electroretinography findings

Separate statistical comparisons of amplitude and implicit time of each ring N1 and P1 waves, mean values of N1 and P1 wave parameters and changes in all rings were statistically significant (Tables 10–13).

3.4. Correlation analyses

Changes in the best-corrected visual acuity and retinal sensitivity were non-significantly negatively correlated (p = 0.652; and r=-0.080). However, changes in the best-corrected visual acuity and local deficit were non-significantly moderately correlated (p = 0.795; and r = 0.480).

Although changes in central macular thickness and local defect were significantly negatively correlated (p = 0,001; and r=-0,644), there was a non-significant negative correlation between changes in central macular thickness and retinal sensitivity (p = 0,332; and r=-0,122).

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The internal retinal layer central ring parameters were very highly

Table 3
Changes of the central macular thickness during therapy (n=33).

Time interval	Central macular thickness (μm)	95 % Confidence interval		P value
		Lower limit	Higher limit	
Baseline	58.5±232.1			
3rd month	273±109.9	25.5	145.6	0.007
6th month	245.5±109.3	63.2	162.9	0.000

Table 4
The C1MTA[†] changes during therapy ($n = 33$).

Time interval	(C1MTA) (μm)	95% CI[*]		*P* value
		Lower limit	Higher limit	
Baseline	349.5 ± 96.4			
3rd month	320.6 ± 101.9	7.3	50.4	0.010
6th month	290.5 ± 86.4	36.8	81.1	0.000

[*] 95% CI-Confidence interval of the difference between values.
[†] C1MTA-The macular thickness average of the central 1 mm area.

Table 5
Changes of the retinal areas designated by ETDRS[a] during therapy.

The retinal areas designated by the ETDRS	Baseline	6th month	P value
Retinal nerve fiber layer central ring	27.4 μm	18.6 μm	0.000
Retinal nerve fiber layer inner ring	31.3 μm	25.8 μm	0.000
Retinal nerve fiber layer outer ring	45.9 μm	35.4 μm	0.000
Ganglion cell layer central ring	19.2 μm	17.6 μm	0.263
Ganglion cell layer inner ring	36.5 μm	34.2 μm	0.038
Ganglion cell layer outer ring	30.0 μm	27.9 μm	0.002
Internal plexiform layer central ring	25.7 μm	24.8 μm	0.678
Internal plexiform layer inner ring	36.1 μm	30.7 μm	0.000
Internal plexiform layer outer ring	28.9 μm	27.3 μm	0.062
Internal plexiform layer central ring	33.8 μm	25.3 μm	0.000
Internal plexiform layer inner ring	43.0 μm	40.0 μm	0.001
Internal plexiform layer outer ring	36.3 μm	32.7 μm	0.000
Outer plexiform layer central ring	34.3 μm	24.9 μm	0.000
Outer plexiform layer inner ring	36.3 μm	32.0 μm	0.001
Outer plexiform layer outer ring	30.4 μm	28.8 μm	0.001
Outer nuclear layer central ring	81.2 μm	72.6 μm	0.177
Outer nuclear layer inner ring	71.8 μm	65.1 μm	0.079
Outer nuclear layer outer ring	58.3 μm	53.0 μm	0.000
Retinal pigment epithelium central ring	47.2 μm	38.5 μm	0.180
Retinal pigment epithelium inner ring	34.0 μm	28.9 μm	0.042
Retinal pigment epithelium outer ring	20.8 μm	20.0 μm	0.366
Internal retinal layer central ring	218.2 μm	184.3 μm	0.000
Internal retinal layer inner ring	250.6 μm	238.9 μm	0.014
Internal retinal layer outer ring	219.2 μm	212.9 μm	0.023
Outer retinal layer central ring	177.7 μm	150.6 μm	0.000
Outer retinal layer inner ring	136.7 μm	124.5 μm	0.028
Outer retinal layer outer ring	107.6 μm	105.2 μm	0.264

[a] Early Treatment Diabetic Retinopathy Study.

Table 6
Changes of the fixation stability data during therapy (n=33).

Fixation stability analysis	Baseline	6th month
Stable	4 (12.1 %)	18 (54.5 %)
Relatively unstable	12 (36.4 %)	10 (30.3 %)
Unstable	17 (39.4 %)	5 (15.2 %)

Table 7
Changes of the fixation localization data during therapy (n=33).

Fixation Localization	Baseline	6th month
Predominantly Central	6 (18.2 %)	19 (57.6 %)
Weakly Central	14 (42.4 %)	11 (33.3 %)
Eccentric	13 (39.4 %)	3 (9.1 %)

Table 8
Changes of the retinal sensitivity data during therapy (n=33).

Time interval	Retinal sensitivity (dB)	%95 Confidence interval		P value
		Lower limit	Higher limit	
Baseline	4.7±3.0	3.6	5.8	
6th month	6.9±3.4	5.7	8.1	0.000

Table 9
Changes of the local deficits during therapy (n=33).

Time interval	Local deficits (dB)	%95 Confidence interval		P value
		Lower limit	Higher limit	
Baseline	−11.6±4.6	−13.2	−9.9	
6th month	−9.4±4.6	−10.9	7.7	0.000

Table 10
Changes of the N1 amplitude values in each ring during therapy (n=33).

	Baseline N1 amplitude value (nv/deg^2)	6th month N1 amplitude value (nv/deg^2)	P value
1st ring	23.1±9.1	25.6±10.4	0.000
2nd ring	20.0±12.7	24.9±12.4	0.000
3rd ring	17.2±5.6	18.9±4.9	0.000
4th ring	11.3±4.5	13.2±5.2	0.011
5th ring	8.8±2.9	10.9±4.7	0.000

Table 11
Changes of the N1 implicit values in each ring during therapy (n=33).

	Baseline N1 implicit value (ms)	6th month N1 implicit value (ms)	P value
1st ring	29.3±3.1	26.8±3.0	0.000
2nd ring	28.1±2.9	26.4±2.8	0.000
3rd ring	26.4±1.5	24.6±3.5	0.000
4th ring	26.0±2.0	25.1±1.9	0.000
5th ring	26.1±1.3	24.7±2.0	0.000

Table 12
Changes of the P1 amplitude values in each ring during therapy (n=33).

	Baseline P1 amplitude value (nv/deg^2)	6th-month P1 amplitude value (nv/deg^2)	P value
1st ring	50.4±36.3	52.4±11.8	0.165
2nd ring	37.2±16.7	43.2±16.2	0.000
3rd ring	30.5±7.4	32.7±7.4	0.000
4th ring	24.3±5.7	28.4±6.3	0.000
5th ring	19.6±4.2	21.6±3.7	0.000

Table 13
Changes of the P1 implicit values in each ring during therapy (n=33).

	Baseline N1 Implicit value (ms)	6th-month N1 Implicit value (ms)	P value
1st ring	48.8±4.8	44.5±3.9	0.000
2nd ring	47.8±4.1	45.5±3.0	0.000
3rd ring	45.7±2.3	44.1±2.4	0.000
4th ring	44.6±2.2	43.2±1.9	0.000
5th ring	44.4±2.0	43.1±1.8	0.000

negatively correlated with changes in retinal sensitivity ($p = 0.001$; and r=-0.870). There was, however, a weak negative correlation between the outer retinal layer central ring parameters and changes in retinal

sensitivity ($p = 0.363$; and r=-0.163).

Despite a significant negative correlation between the internal retinal layers central ring and changes in local defect ($p = 0.597$; and r=-0.660), there was a weak negative correlation between the outer retinal layer central ring and changes in local deficit ($p = 0.852$; and r=-0.230).

Multifocal electroretinography assessment revealed significantly moderate correlation between changes in N1 amplitude 1st ring and retinal sensitivity ($p = 0.001$; and $r = 0.640$). There was, however, a non-significant weak correlation between changes in N1 amplitude 4th ring and retinal sensitivity ($p = 0.558$; and $r = 0.106$). Moreover, there was a non-significantly negative correlation between changes in the 1st ring N1 amplitude and the best-corrected visual acuity ($p < 0,001$; and $r = 0.321$), but a significantly highly negative correlation between changes in the 1st ring N1 amplitude and central macular thickness ($p < 0.001$; and r=-0.764). There was a very weakly negative correlation between changes in the 1st ring N1 implicit time and the best-corrected visual acuity ($p = 0.062$; and r=-0.021).

Although there was a significantly moderate correlation between changes in P1 amplitude and the best-corrected visual acuity ($p < 0.001$; and $r = 0.321$), changes in the 4th ring P1 amplitude and the best-corrected visual acuity were non-significantly weakly correlated ($p < 0.126$; and $r = 0.130$). Further, although there was a very weak negative correlation between changes in P1 implicit time and the best-corrected visual acuity ($p = 0.156$; and r=-0.121), changes in the 4th ring P1 implicit time and the best-corrected visual acuity were statistically non-significant and very weakly negatively correlated ($p = 0.052$; and r=-0.120).

On the other hand, there was a significantly high negative correlation between changes in P1 amplitude and central macular thickness ($p < 0.001$; and r=-0.791). Similarly, the correlation between the 2nd and 4th ring P1 amplitudes and changes in central macular thickness was significantly highly negatively correlated, respectively [($p < 0.001$, r=-0.678); and ($p < 0.001$, r=-0.694].

4. Discussion

In the current study, statistically significantly improved best-corrected visual acuity and optical coherence tomography parameters were observed in neovascular age-related macular degeneration patients as of the 3rd month following the onset of aflibercept therapy. This progressive improvement was achieved by an average of 4.5 intravitreal aflibercept injections. However, records in electrophysiological studies generally showed improvement starting from the 6th month after therapy. These results demonstrated that macular electrophysiological improvement occurs over the long-term following anti-vascular endothelial growth factor therapy. It has also been revealed that the global retinal functional status does not always worsen. On the contrary, some gradual improvement may still occur after anti-vascular endothelial growth factor therapy over a longer period of time.

A considerable number of studies evaluating the role of macula with ranibizumab have been published in the literature [11,12]. However, there is a very limited number of studies investigating anatomical and functional improvements after intravitreal aflibercept in patients with neovascular age-related macular degeneration. Aflibercept is a 115 kDa recombinant fusion protein developed by bioengineering that binds to an extracellular portion of the vascular endothelial growth factor-1 and 2 receptors. This agent, which was approved by the Food and Drug Administration with the VIEW-1 and VIEW-2 studies in 2011, inhibits the vascular endothelial growth factor A and B, as well as the placental growth factor. It binds to the vascular endothelial vascular growth factor-A with an affinity 100 times that of bevacizumab and ranibizumab [13].

In a retrospective analysis of the VIEW-1 and VIEW-2 studies performed by Sarwar et al. [14] on optical coherence tomography findings, no major differences were found as compared to 12th-month changes in central macular thickness between aflibercept and ranibizumab cases (VIEW-1 aflibercept group-120.2, ranibizumab group-116.8; VIEW-2 aflibercept group-145.5, ranibizumab group-145.5). A comparable proportion of 3.3 % and 3.2 % of arterioembolic events were observed in the aflibercept and ranibizumab groups, respectively. As regards changes in the best-corrected visual acuity and central macular thickness, the VIEW-1 and VIEW-2 studies showed a statistically significant relationship consistent with the current study.

Jaggi et al. [15] reported long-term outcomes on best-corrected visual acuity following aflibercept treatment with a treat-and-extend regimen without a loading phase in 82 naive neovascular age-related macular degeneration patients in a retrospective study. The average duration of the follow-up period was 3.5 ± 0.65 years, and the average number of injections among patients who had received $\geq$2 years of therapy was 19.2 ± 9.0. Initially, there was a statistically significant improvement in best-corrected visual acuity (ETDRS letters) from 51.9 ± 25.2 at baseline to 63.7 ± 17.7, 61.7 ± 18.5, and 62.4 ± 19.5 at 1, 2, and 3 years, respectively ($p < 0.0001$ for all). Although improved best-corrected visual acuity remained higher at 58.5 ± 24.3 after 4 years, the difference was not statistically significant when compared to baseline ($p = 0.22$). Improved visual acuity was accompanied by a significantly decreased central subfield thickness, which decreased from 387.5 ± 107.6 μm at baseline to 291.9 ± 65.5 μm at 1 year and remained significantly lower until 4 years at 289.0 ± 59.4 μm. The gradual decline in visual acuity from years 1–4 could be attributed primarily to the dry age-related macular degeneration component and fibrosis, rather than recurrent choroidal neovascularization activity. These findings, which were consistent with previous large clinical trials, suggested that initial vision gains could be maintained with an appropriate treatment approach after long-term therapy, even without an initial loading phase. In the current study, statistically improved visual acuity and, in addition to other optical coherence tomography parameters, decreased central macular thickness was observed as of the 3rd month after starting aflibercept therapy. The current study, unlike the above one, included an initial loading phase for all patients. Furthermore, patients were prospectively followed up on a monthly basis for at least six months, with a mean number of injections of 4.5 resulting in both retinal anatomical and functional improvements.

Unsal et al. [16] conducted a study in which aflibercept was administered to 43 eyes of 43 patients resistant to ranibizumab and bevacizumab. They recorded increased best-corrected visual acuity from 1.14 ± 0.51 to 0.84 ± 0.59 logMAR and decreased central macular thickness from 402.6 ± 196.7 μm to 276.7 ± 47.9 μm in the 3rd month. Correspondingly, the current study showed significantly improved visual acuity and central macular thickness findings as early as 3 months following intravitreal aflibercept therapy, which may be ascribable to early improvement in intra- and sub-retinal neural transmission, and therefore enhanced visual performance.

Minami et al. [17] reported a significant decrease in central macular thickness during the 6th and 12th months when comparing typical visual acuity, contrast and functional vision and optical coherence tomography parameters in age-related macular degeneration patients (mean age 74 ± 8.76 years) following aflibercept therapy. There was also a significant decrease in volume width of the internal retinal layers in the 6th month, but not in the 12th month. On the other hand, the outer retinal layer volume width decreased significantly in the 6th and 12th months. There was a moderate correlation between decrease in volume width of the outer retinal layer and increase in the best-corrected visual acuity. But, decrease in volume width of the internal retinal layers and change in central macular thickness was non-significantly weakly correlated. Contrastingly, visual function was significant in all ring thicknesses and central and inner rings of the outer retinal layer in the current study. Moderate correlation between increase in visual function and decrease in central macular thickness was observed. Also, unlike the above study, visual function parameters were; highly correlated with decrease in the internal retinal layers and central

area parameters, moderately correlated with decrease in the inner ring parameters, and very highly correlated with decrease in the outer ring parameters. On the other hand, increase in visual function was highly correlated with decrease in the outer retinal layer central ring parameters, and weakly correlated with decreases in the inner and outer ring parameters, which corresponded to Minami study. The increase in visual function was strongly correlated with decrease in the central ring parameters of the outer retinal layer and weakly correlated with decrease in the inner and outer ring parameters, which also corresponded to Minami study. All of these different outcomes for improving neuroretinal function starting in the 6th month basically involve neurophysiological enhancement of photoreceptors in the long-term following intravitreal afibercept therapy.

Microperimetry is capable of accurately measuring retinal sensitivity and fixation stability in macular diseases [9]. In one study, following central serous chorioretinopathy, retinal sensitivity remained poor despite normal macular structural appearance and normal visual acuity [18]. Structural and morphological changes have been shown to be correlated with microperimetry visual function in macular diseases such as age-related macular degeneration [19,20], in which it has been found that retinal sensitivity decreased as predicted with increasing thickness. As microperimetry provides more predictive and quantitative functional measurements for the best-corrected visual acuity and optical coherence tomography thickness, this is considered to be the most useful visual acuity functional test [21]. Barboni et al. [22], reported improved retinal sensitivity in patients with age-related macular degeneration relative to healthy individuals.

In the study conducted by Sakai et al. [23] in which 25 patients with an average age of 70 were examined following 12 months of intravitreal aflibercept therapy, there were statistically significant improvements in the best-corrected visual acuity and decreases in central macular thickness and foveal sensitivity. There was, however, no significant improvement in local deficit. Similar to the Sakai study, the current study also reported significant improvements in the best-corrected visual acuity and decreases in central macular retinal sensitivity. But, unlike the Sakai study, the decrease in local deficit was statistically significant in the current study. While more patients were present in the current study relative to the Sakai study, follow-up period of the current study was shorter, which is in one of its limitations that might have hindered exploitation of the long-term changes in neuroretinal function on the whole. The discrepancy in the statistical significance of changes in local deficit may be due to the use of microperimetry-1 in the current analysis.

Sulzbacher et al. [24] studied the relationship between retinal morphology and sensitivity in 22 naïve neovascular age-related macular degeneration following intravitreal aflibercept therapy using spectral-domain optical coherence tomography and microperimetry-1. While the best-corrected visual acuity letters increased from 51.6 ± 14.9 to 60.4 ± 13.1 in the 3rd month, this increase levelled to 60.2 ± 14.9 in the 12th month with statistically non-significant improvements. There was also a statistically significant increase from the 3rd month and a non-significant increase from the 12th month in microperimetry-1 data evaluation. As in the above study, the current study observed a statistically significant increase in retinal sensitivity relative to baseline values during the 6th month. This indicates a gradual improvement in neuroretinal function that is expected to continue over the longer term.

The study published by Cho et al. [25] in which 39 neovascular age-related macular degeneration patients were examined for morphological and functional improvements following ranibizumab treatment, reported significantly increased mean visual acuity and significantly decreased central macular thickness at the end of 12 months. However, the increase in mean retinal sensitivity during the 3rd month was not statistically significant at all, but was still statistically significant during the 6th and 12th months. Moreover, Parravano et al. [26] analyzed morphological and functional effects of the 2-year ranibizumab therapy, which showed a non-significantly improved visual acuity after 24 months. There was, however, a significant decrease in central macular thickness and increase in mean retinal sensitivity. In the same study, it was suggested that despite the decrease in visual acuity and retinal thickness that began in the 4th week, retinal sensitivity could take 24 weeks to reflect improvement. While consistent findings from Cho et al. [25] were observed in the current study, these findings were not consistent with Parravano et al. [26]

Sulzbacher et al. [27] reported a significant increase in retinal sensitivity in serous pigment epithelial detachment compared to fibrovascular pigment epithelial detachment following monthly ranibizumab therapy. Finally, they argued that the absence of a significant increase in visual function was due to the suppression of the endothelial vascular growth factor, since the development of epithelial pigment detachment plays an important role in tissue damage. Bolz et al. [28], showed a significant increase in mean visual acuity and a significant decrease in central macular thickness after analysis of morphological and functional aspects in 29 eyes receiving ranibizumab. However, they reported a non-significant change in central retinal sensitivity. Although there was a significant correlation between visual function and baseline central macular thickness, no significant correlation was found during the follow-up months. In the same study, it was suggested that despite the marked decrease in intraretinal and subretinal fluid and improvement in visual acuity following the 1st intravitreal injection, the increase in the number of injections seemed to have less impact at all. In comparison to the above results, the current study revealed a significant increase in retinal sensitivity during 6th month. Although there was a moderate correlation between increase in visual acuity and decrease in central macular thickness, a non-significantly weak negative correlation between increases in visual acuity and retinal sensitivity was observed. Again, a non-significantly negative correlation between central macular thickness and retinal sensitivity was observed during the 6th month. As a result, while changes in visual function, optical coherence tomography and microperimetry-1 were consistent with Bolz et al. [28] different patterns of correlation were observed. This discrepancy may have been associated with a longer follow-up time, as well as the use of different anti-vascular endothelial growth factor (aflibercept) agent which has a 100-fold affinity to vascular endothelial growth factor-A compared to ranibizumab used in Bolz et al. [28]

Munk et al. [29] analyzed the functional results from fundus fluorescein angiography and microperimetry-1 during 12-month follow-up following anti-vascular endothelial growth factor therapy in 61 naive neovascular age-related macular degeneration, and revealed significant increases both in visual acuity and mean central retinal sensitivity, but not significant increases in fixation stability. They also reported negative correlation between retinal sensitivity and visual acuity. Similarly, the current study demonstrated significant improvement regarding retinal sensitivity. Changes in positive fixation stability during the 6th month were also statistically significant. Furthermore, a non-significantly weak negative correlation between visual acuity and increased retinal sensitivity was observed.

Multifocal electroretinography records local electrophysiological responses in different retinal regions [10] that consist of bi-phasic fluctuation with a negative decrease (N1) followed by a positive peak (P1). Usually, there is a second negative wave called N2. N1 is often assumed to be produced by the photoreceptors, while P1 is produced by the Müller and bipolar cells. [30,31]. The relationship between neuroretinal functions and both anatomical and visual results in neovascular age-related macular degeneration patients is highly essential. Patama et al. [32], examined 26 patients undergoing multifocal electroretinography after a single dose of intravitreal ranibizumab injection. Comparatively higher central amplitude values were found in multifocal electroretinography of a group with the highest visual acuity, but there was no difference between groups in implicit times. Further, there were significant changes in the 3rd month P1 and N1 implicit times relative to baseline and 1st month, but there was no significant difference in the

amplitude values. In the light of these observations, it was concluded that intravitreal ranibizumab did not completely provide neuroretinal healing, although a structural decrease in macular thickness could be observed. Conversely, in the current study, the amplitude and implicit values of P1 and N1 waves increased dramatically in tandem with the increase in visual acuity and improvement in central macular thickness.

Moschos et al. [33] recorded no change in the results of multifocal electroretinography despite the decrease in macular edema during the 3rd month following a single dose of intravitreal bevacizumab injection. According to this study, edema-related atrophy in the photoreceptor layer and retinal pigment epithelium which has undetectable vision effects may have been the underlying reason. While macular thickness did not affect visual acuity and electrophysiological responses during the initial phase of the disease, the same study reported a linear correlation between visual acuity and P1 amplitude in the following months. Campa et al. [34] recorded a correlation between multifocal electroretinography and visual acuity and central macular thickness, respectively. In addition, Maturi et al. [35], reported a correlation between decrease in central macular thickness and multifocal electroretinography 1st ring responses following anti-vascular endothelial growth factor therapy. However, Karanjia et al., [36] reported no statistically significant difference between multifocal electroretinography values taken before and two weeks after a single dose of bevacizumab injection. With the exception of Moschos et al. [33], where patients were followed up for 12 months, patients were followed up to a maximum duration of 3-month therapy in all aforementioned studies. Moreover, Feigl et al. [37], indicated that changes in neurophysiology may take time after three months of follow-up in patients who are still receiving therapy.

In fact, very few studies have evaluated multifocal electroretinography results following intravitreal aflibercept therapy. Certainly, functional improvement of the retina was observed in the current study from the 1st month multifocal electroretinography and the 3rd month microperimetry findings, and improvement continued until the 6th month. In conclusion, the conflicting findings of electrophysiological responses in the literature may be explained by the disparity not only in the number of cases and initial characteristics, but also in the follow-up periods of the studies. The current study, however, revealed statistically significant functional changes with respect to multifocal electroretinography values one month after aflibercept therapy, in which the increase in amplitude and the decrease in implicit time were considered to be functional improvements. Since neovascular age-related macular degeneration mainly affects fovea and parafovea, the current study focused in particular on 1st and 2nd ring values.

De Oliveira Dias et al. [38] evaluated changes in visual acuity, optical coherence tomography and electrophysiological studies after ZIV-aflibercept injection in neovascular age-related macular degeneration. They revealed significantly improved visual acuity and significantly decreased central macular thickness and total macular volume during the 26th week. There was also a significant increase only in the 1st ring of N1 and P1 wave amplitudes. No significant changes were observed in N1 and P1 wave implicit time. Conversely, the current study revealed a moderate correlation between improved visual acuity and decreased central macular thickness. Analysis of electrophysiological changes in the 4th ring to determine the toxic effect of aflibercept therapy on a healthy retina showed no degradation in P1 and N1 wave values. On top of that, there have been occasional changes. As a result, the authors suggest that aflibercept does not appear to have a major toxic effect on the retina.

One of the advantages of the current study is the simultaneous performance of multifocal electroretinography and microperimetry. There are very few studies, however, investigating the above-mentioned procedures in the literature. Wu et al. [39] found no association between implicit time and visual acuity in the comparative study between 60 age-related macular degeneration patients and 44 control subjects. However, age-related macular degeneration patients were associated with significantly reduced N1 and P1 wave amplitudes, significantly prolonged P1 implicit time and decrease in retinal sensitivity. Another study performed by Reinsberg et al. [40], evaluated clinical values of multifocal electroretinography and microperimetry in macular function following monthly ranibizumab injection in 20 patients with age-related macular degeneration. Improved visual acuity, decreased central macular thickness, increased P1 wave amplitude and scotoma region were all detected 12 months after therapy. Although there was a positive correlation between improved visual acuity and scotoma area width, there was a negative correlation between visual acuity and P1 wave amplitude. There was also no correlation between change in P1 amplitude and decrease in central macular thickness. It was therefore proposed that multifocal electroretinography would better represent practical improvements rather than changes in the best corrected visual acuity and optical coherence tomography. Multifocal electroretinography enables us to understand the behavior of bipolar cells and the function of certain photoreceptors and internal retinal cells. Thus, improvements in multifocal electroretinography and P1 amplitudes following intravitreal injection in neovascular age-related macular degeneration suggest the presence of different cellular activities. This may indicate the potential value of the retinal sensitivity measured by microperimetry in the assessment of macular function compared to the best-corrected visual acuity. Contrary to Reinsberg study [40], the current study revealed significantly increased visual acuity. However, corresponding results were obtained with respect to a significantly decreased central macular thickness. When comparing the two studies on changes in microperimetry, the main difference was that the current study did not involve measurements of scotoma width, which have been studied in only a few studies [28,29,41]. Although the increase in P1 amplitude was not statistically significant in the Reinsberg study, a significant increase in P1 amplitude was revealed in the current study. In addition, the current study revealed a moderate positive correlation between increase in visual acuity and decrease in central macular thickness. There was, however, significant negative correlation between baseline values of the 1st ring P1 and visual acuity and the 6th month values of the 1st ring P1 and the central macular thickness. As revealed in the current study, Reinsberg et al. [40] reported significant negative correlation between P1 wave amplitude and increase in visual acuity. But, no correlation between P1 amplitude and change in central macular thickness was reported.

5. Conclusions

The current study has shown that visual acuity and optical coherence tomography parameters alone may, to some extent, be inadequate for both morphological and functional assessment of the retina. Changes in optical coherence tomography parameters reflecting anatomical and structural integrity, and changes in microperimetry and multifocal electroretinography reflecting retinal functionality and neurophysiology improved significantly following intravitreal aflibercept therapy in neovascular age-related macular degeneration. Multi-center studies with a larger study population and longer follow-up periods are required to provide more comprehensive and further reliable information on this subject.

Ethics approval and consent to participate

All procedures conducted in studies involving human subjects were in compliance with the ethical principles of the Institutional and National Research Committee and the Helsinki Declaration of 1964 and its corresponding modifications or equivalent ethical standards.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

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Declaration of Competing Interest

The authors report no declarations of interest.

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