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ORIGINAL PAPER

Long-term full-field and multifocal electroretinographic changes after treatment with ranibizumab in patients with diabetic macular edema

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Abstract

Purpose To investigate changes in macular and panretinal neuroretinal functions by electroretinographic examinations in eyes with diabetic macular edema (DME) treated with intravitreal ranibizumab.

Material and methods Sixty-four patients with DME were included in this prospective study. Patients were treated with ranibizumab injection according to the PRN regimen for over 12 months. Before treatment, all patients underwent fundus fluorescein angiography, optical coherence tomography (OCT), best-corrected visual acuity (BCVA) assessment, full-field (ff-ERG), and multifocal electroretinography (mf-ERG). In monthly visits, BCVA and OCT were performed. Besides, mf-ERG recordings were obtained at months 3, 6, 9, and 12, and ff-ERG was performed at month 12.

Results Fifty-eight patients completed the study. The mean age was 61.1 ± 8.5 (39–80) years. The mean number of injections was 6.19 ± 1.9. The decimal BCVA improved from 0.30 to 0.45 during the 12-month follow-up ($p < 0.05$). Macular thickness decreased from 413.5 µm to 329.5 µm ($p < 0.05$). The mf-ERG recordings in the central macular region showed improvements N1 and P1 amplitudes at months 9 and 12. There was a positive correlation between the baseline central ($p < 001$; r: − 0.378 and $p < 0.05$; r:-0.335, respectively), the second ring ($p < 0.05$; r: − 0.260 and $p < 0.05$; r: − 0.270, respectively) P1- and N1-wave amplitudes, and the BCVA at month 12. Full-field ERG recordings showed that peripheral neuroretinal responses were maintained or improved at month 12. Statistically significant improvements in BCVA and macular thickness were observed at all follow-up visits.

Conclusion Multifocal electroretinographic recording started to improve 6 months after the beginning of intravitreal ranibizumab treatment in eyes with DME. This improvement was significant at months 9 and 12. A significant improvement in ff-ERG was observed at month 12.

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Keywords Diabetic macular edema · Electroretinography · Neuroretinal functions · Ranibizumab

Introduction

Diabetic macular edema (DME) is the most common cause of vision loss in diabetic patients. Following a 15-year established DM, the prevalence of DME was reported to be approximately 20%, 25%, and 14% in patients with type I DM, insulin users with type II DM, and non-insulin users with type II DM, respectively [1]. Meanwhile, the mean rate of DME in all diabetic patients was 6% [2].

While macular edema generally occurs via fluid accumulation in the extracellular region, it may also develop as a result of the potassium channels on the hypoxia-stimulated cell membrane being affected and the resulting intracellular potassium accumulation, followed by the development of intracellular edema via increased intracellular osmotic pressure. This cascade of events leads to swelling of glial cells, edema, and cyst formation [3]. The resulting intraretinal edema may lead to functional impairment in retinal Müller and adjacent neural cells. Consequently, intraretinal synaptic connections among the retinal neural cells and photoreceptors may be disrupted [4].

VEGF-A is involved in vascular permeability in the DME physiopathology [5]. Ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA, USA) has been shown to provide a visual and anatomic improvement in the treatment of DME [6–8]. VEGF inhibition has been reported to increase the ganglion cell apoptosis and the neuronal cell apoptosis on the inner nuclear layer, primarily in the amacrine and bipolar cells [9]. Electrophysiological tests can be beneficial to objectively show whether an unfavorable effect occurs on the retinal neuronal cells concerning anti-VEGF treatment as well as intercellular edema among the retinal macular layers. The electrophysiological investigation can also demonstrate the functional recovery in the retinal cells after the resolution of macular edema.

The multifocal ERG (mf-ERG) recording technique was developed for the topographic measurement of the retinal electrophysiological activity [10]. Mf-ERG can assess the macula functions at more than 60 locations within a period as short as 8–10 min and exhibit the responses from the inner and outer retina and show them topographically. A small portion of the mf-ERG response results from the cone receptors and consists predominantly of the on and off bipolar cell responses [11]. Second-rank kernel analysis showed that inner retinal layers had a substantial contribution [12]. The typical waveform of mf-ERG is a biphasic wave that starts with a negative deviation followed by a positive peak. Three wave forms are, respectively, called N1-, P1-, and the N2-wave. There is evidence on the contribution of the cells, involved in the formation of the a-wave on full-field cone ERG, to the formation of the mf-ERG N1-wave. Similarly, the P1-wave contains the responses of the cells that make up the b-wave and oscillatory potentials. Thus, it was demonstrated that the N1-wave results from the cone cells and the P1-wave from the bipolar cells [13, 14]. Mf-ERG may objectively reveal to what extent the retinal cells are affected in diabetic macular edema. Therefore, mf-ERG may provide comprehensive data on the change in macular function during the DME course in a way to show the health of the middle and the outer retinal layers [15–25]. So, electrophysiological assessment by mf-ERG provides a more global health assessment of the macula compared to visual acuity which reflects a retinal function at a 1-degree angle [26].

In patients with long-term diabetes, significant changes are reported in ERG even if there is no retinopathy [27, 28]. Full-field ERG is a well-defined technique to measure the global retinal function and may reveal deterioration in current retinopathy or unfavorable effects of intravitreal agents on retinal cells [29]. The changes in peripheral retinal and macular function by electrophysiological assessment in patients with DME during treatment with intravitreal ranibizumab have rarely been studied [30–32]. Thus, relevant information remains to be deficient.

In our study, we aimed to investigate the changes in macular and global retinal function by mf-ERG and ff-ERG recordings in eyes with DME treated with long-term intravitreal ranibizumab. We aimed to reveal any retinal toxicity or improvement in retinopathy level due to long-term anti-VEGF treatment with ff-ERG testing.

Material and methods

Sixty-four eyes with DME were included in this prospective study. The diagnosis of DME was established by binocular stereoscopic fundus examination, optical coherence tomography (OCT) examination and fundus fluorescein angiography (FFA).

Institutional Clinical Trials Ethics Committee reviewed and approved the study. Informed consent was obtained from all patients after an explanation of the nature and possible consequences of the study. The study adhered to the tenets of the Declaration of Helsinki.

All patients underwent a routine ophthalmological examination before inclusion into the study. BCVA was measured with ETDRS chart. All visual acuity results were transformed to the common logarithm of the minimum resolution angle (Log MAR). Intraocular pressure was measured using applanation tonometry. Fundus examination was performed using indirect non-contact funduscopy. At baseline, color fundus photography and FFA (Zeiss Visucam 500, Carl Zeiss Meditec AG, Oberkochen, Germany) were performed (Fig. 1a). Using the OCT (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany) imaging, the central macular thickness and the concomitant vitreoretinal interface disorders were recorded (Fig. 1b). Once segmentation defects were determined, the correction was performed by manually moving the reference lines over the internal limiting membrane and the Bruch membrane, and the thickness between the new reference lines was measured after using the automatic centralizing program of the device.

The patients received intravitreal ranibizumab injections (Lucentis; Genentech, Inc., South San Francisco, CA, USA) consecutively for the first three months at 1-month intervals; only one additional dose was given if DME persisted or recurred during monthly follow-ups. The retreatment criteria were recurrence or persisted of macular edema was defined as an increase in macular thickness by more than 20% compared to the last examination after an initial improvement which causes deterioration of visual acuity and/or newly diagnosed fovea-involving intraretinal or subretinal fluid causing visual deterioration. All intraocular injections were performed in the operating theater. Before injection, local anesthesia (0.5% proparacaine hydrochloride, Alcaine, Alcon) and 5% povidone-iodine sterilization procedures were performed. 0.5 mg/0.05 ml ranibizumab was injected with a 30 gauge needle 3.5 mm from the limbus in the superior temporal region. After the procedure, patients were prescribed antibiotic drops. At baseline, mf-ERG and ff-ERG was performed. Subsequently, mf-ERG recordings were obtained at months 3, 6, 9, and 12, and ff-ERG was performed at month 12. None of the eyes had previously received any intravitreal treatment or photocoagulation. Eyes with media opacity, any retinal vascular disorder other than DME, ischemic maculopathy, tractional DME, previous posterior segment surgery, glaucoma, optic nerve pathology, and previous or new inflammatory pathology were not included in the study. Failure to regularly attend the control examinations, inability to cooperate during mf-ERG and ERG, declaration of inability to continue with treatment, endophthalmitis, retinal detachment, intravitreal hemorrhage, complications associated with intravitreal injection, systemic complications due to treatment agent were set as exclusion criteria. All patients had non-proliferative diabetic retinopathy (PDR). The patients with PDR were not included in the study. Both type 1 and type 2 patients were included (Table 1). The patients with BCVA between 0.1 and 0.8 and center involving DME with CMT greater than 300 μm were included in the study. Only one eye was treated and included in the study. The patients who required the treatment in the fellow eye during the follow-up were not excluded from the study. Treatment was open label. The evaluation of the eyes was not blind.

All mf-ERG and ff-ERG procedures were conducted by the same technician using the same device (Metrovision Monpack 3, Metrovision, France). A representative test for mf-ERG and ff-ERG from our laboratory is provided in Figs. 1 and 2. Following the obtaining recordings, care was taken to comply with the recent ISCEV standards [33, 34]. Before the procedure, maximum pupillary dilatation was achieved using 1.0% tropicamide. The procedure was performed, correcting the refraction defect based on 33 cm viewing distance. During the conduct of the procedure, ERG-jet electrode was used as the active electrode. ERG-jet electrode was placed on the cornea following one drop of 0.5% proparacaine HCl. Before placement of the ground electrode and the reference electrode, the relevant skin site was cleaned and wiped with alcohol to clean the superficial skin layer and the

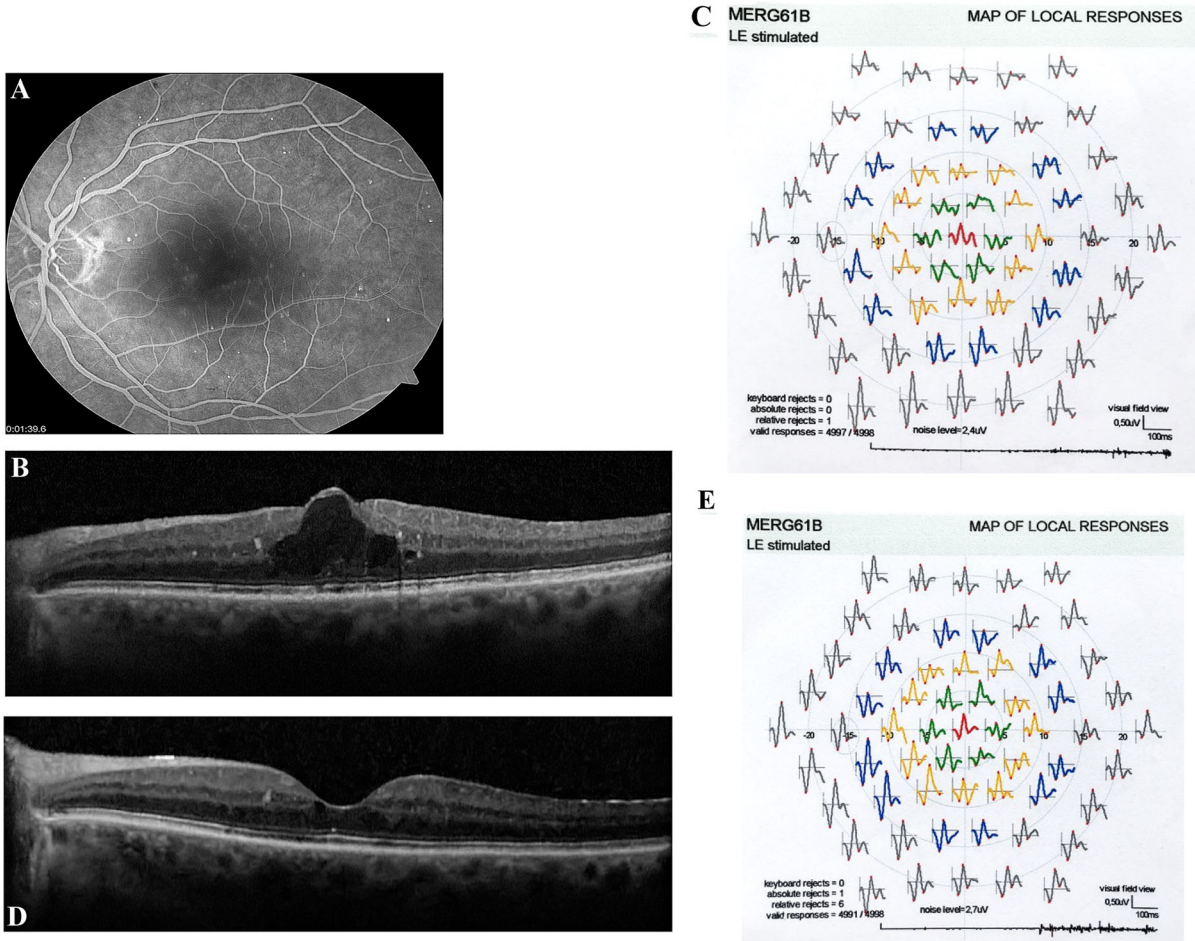

Fig. 1 A representative case was shown for presentation of our laboratory mf-ERG recordings as well as OCT images of the same eye. The patient was a 71-year-old female with 24 years type-2 diabetes mellitus. Her baseline HbA1C was 7.2. Her baseline Snellen equivalent BCVA was 0.25 and improved to 0.63 at month 12. **a** Fundus Fluorescein Angiography shows non-proliferative diabetic retinopathy and fluoresceine leakage from multiple peri foveal microaneurysms in the early phase (1 min 39 s). **b** Baseline OCT of the left eye in a 71-year-old female with naive DME. **c** Map of local responses of baseline mf-ERG recording in the same eye. **d** Shows improvement in central macular thickness in OCT at month 12. **e** Shows improvement in map of local responses of mf-ERG recording at month 12

fatty layer that is known to have low electrical conductivity. Subsequently, the ground electrode was placed slightly over the supraorbital edges at the midline of the forehead, and the reference electrode was put onto the temporal region, 1 cm ahead of the outer canthus. Recording of the signal was achieved by combining the electrodes via the connection box. The eye, which did not undergo imaging, was closed and the patient's jaw was placed in the chin protector. During imaging, fixation was monitored using an infrared camera.

In accordance with the ISCEV criteria, MERG61B test was conducted for mf-ERG. On the monitor screen, an image pattern was used, which was adjusted to form a signal consisting of 61 hexagons of equal size and recordings from 61 sites of the retina were established within approximately 5 min. Screen resolution was set at 1024 × 768. Horizontally, a ± 30-degree area and vertically, a ± 24-degree area was stimulated. The stimulus frequency was 17 Hz; the luminance was 100 candelas per square meter (cd/m^2). Ground illumination was set at 30 cd/m^2. The electrical activity that was present upon the absence of stimulus during the test, the noise level was recorded. Results with a noise level > 5 μV were not included in the assessment. Imaging was repeated when artifact

Table 1 Demographic characteristics of the patients

Characteristics	
Gender (Male/female) (n)	34/24
Mean age (min-max)	61.1±8.5 (39–80) years
Laterality (right/left) (n)	31/27
Mean number of intravitreal injections	6.19±1.9 (4–11)
Follow-up (month)	12
Lens status (Pseudophakia/Phakia)	9/49
Baseline best-corrected visual acuity	0.30±0.18 (0.8–0.1)
Final best-corrected visual acuity	0.45±0.14 (1.0–0.05)
Baseline central macular thickness	413.5 (183–918) μm
Final central macular thickness	329.5 (155–647) μm
Type of DM (type I/type II)	8/50
Duration of DM (after diagnosis)	16.16±7.02 (6–26) years
Fasting glucose level*	164.3±45.4 mg/dL
HbA1C (%)*	7.67±1.4 (5.76–13.14)
Total cholesterol*	180.4±24.6 (130.1–260.0)
Triglyceride*	147.8±46.4 (69.2–217.4)
HDL*	40.9± 11.3 (21.5–58.5)
LDL*	113.8±21.5 (85.8–156.3)
VLDL*	30.4±8.5 (13.84–43.48)

*Baseline serum levels

occurrence was observed. Test results with a loss of attention and a total number of rejected stimuli 20% more than the total number of stimuli were not included in the trial. Concentric ring analysis was performed. For the analysis, the amplitude and implicit time of the N1-wave, and P1-wave of the "first-line kernel" wave in each ring were calculated. For concentric ring analysis, according to fixation, the first ring contained the 0–5-degree area (fovea), the second ring contained the periphery of the 5–10-degree (parafovea), the third ring contained the periphery of the 10–15-degree area, and the fourth ring contained the periphery of the 15-degree area. For the whole ring analysis, the mean amplitude (nanovolt) and the implicit time (milliseconds) were recorded. From the data obtained, the amplitude and implicit time values of the N1- and P1-waves were statistically compared individually for each ring.

For ff-ERG recordings, scotopic (dark adapted) and photopic (light adapted) ERG recordings were obtained. Dark-adapted ERG recordings were obtained after 20 min of dark adaptation, and light-adapted responses were obtained after 10 min of light adaptation. Combined rod-cone responses were obtained using a single white flash stimulus (3 cd/s/m^2) to the dark-adapted eye. Light-adapted responses were obtained using a single white flash (500 cd/s/m^2) as stimulus, and background luminance was 30 cd/s/m^2. 30-Hz light-adapted flicker ERG was also recorded using a white stimulus (500 cd/s/m^2), with 30 stimuli per second. Oscillatory Potentials were not recorded.

Baseline values for BCVA, IOP, OCT measurements, P1-, and N1-waves of mf-ERG were compared to values obtained at months 3, 6, 9, and 12. All numerical data were expressed as means and standard deviations (SD). Normality of the data was evaluated using the Shapiro–Wilk test. For statistical evaluation, SPSS (Statistical Package for Social Science, 17.0 Worldwide Headquarters SPSS Inc.) was used. General Linear Model (ANOVA for repeated measures) and the paired sample t-test were used for comparing the visual acuity, intraocular pressure, macular thickness, mf-ERG and ff-ERG results to baseline values. Bonferroni correction was performed due to the presence of repeated measurements. Due to Bonferroni correction, statistical significance was accepted as 0.01 for repeated measures such as CMT, BCVA, IOP, and mf-ERG measurements. Pearson's bivariate correlation analysis was used to assess the correlation between baseline and final data. Regression analysis was performed to generate a predictive model for

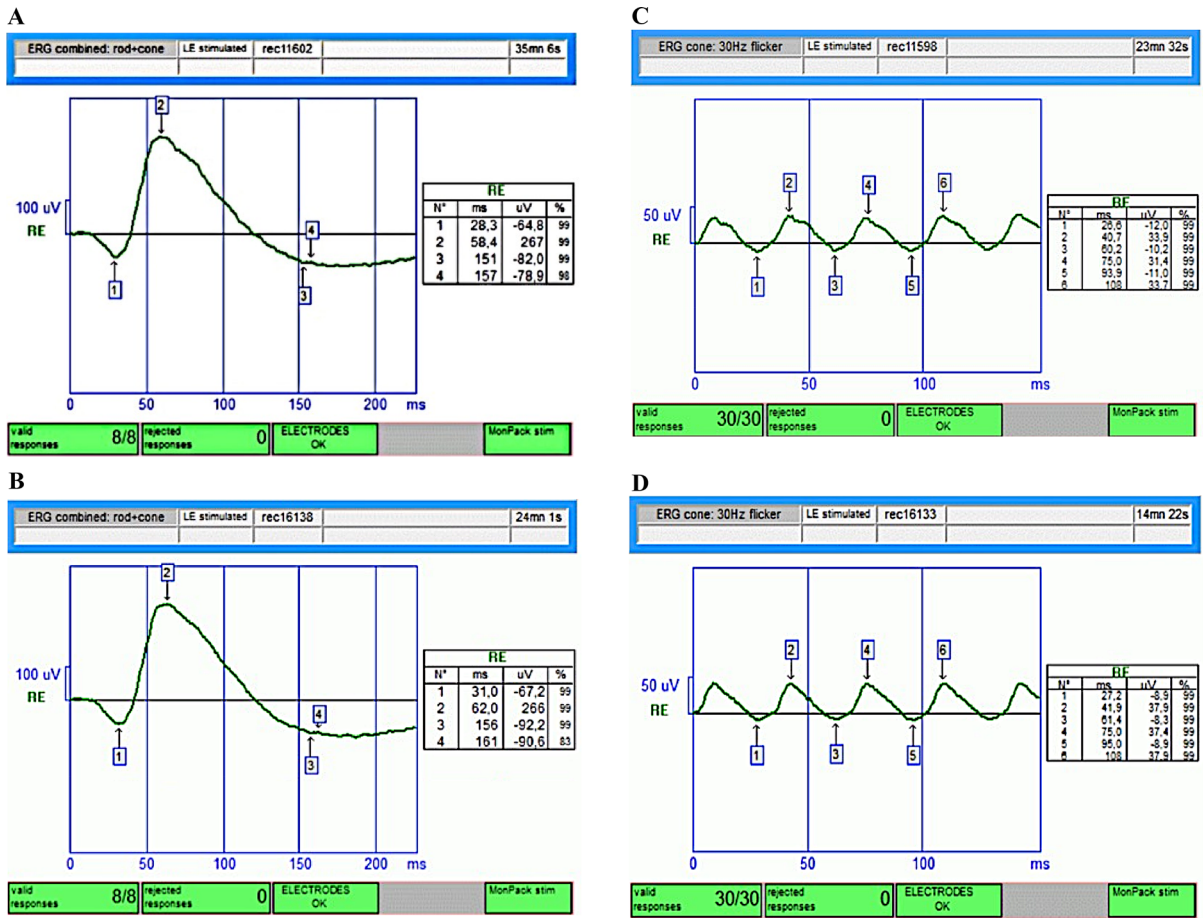

Fig. 2 Representative ff-ERG recordings from our laboratory were shown. **a** Baseline ff-ERG combined rod cone response of the same patient (recorded from the left eye) in Fig. 1. **b** Final ff-ERG combined rod cone response in the same eye. **c** Baseline ERG cone 30 Hz Flicker Response in the same patient (left eye). **d** ERG cone 30 Hz Flicker Response at month 12 in the same eye

anatomical and functional results. A p-value < 0.050 was considered significant for ff-ERG results.

Results

Six patients were excluded from the study due to reasons such as the inability to regularly attend the visits, other health issues, and non-compliance with the ERG procedures. The remaining 58 patients (34 M and 24 F) completed the 12-month follow-up. The mean age was 61.1 ± 8.5 (39–80) years. The mean number of intravitreal ranibizumab injections was 6.19 ± 1.89 (4–11). Baseline characteristics are given in Table 1. Data on visual acuity and CMT are presented in Table 2. Mean CMT and LogMAR BCVA at months 3, 6, 9, and 12 showed statistically significant improvements (Table 2). No statistically significant change was observed in intraocular pressure during the follow-up with baseline value of 14.8 ± 0.38 and final value of 14.0 ± 0.32 (p: 0.08).

Multifocal electroretinography findings

First Ring (0–5°): Mean baseline N1 amplitude showed nonsignificant increase at months 9 and 12 after Bonferroni correction (p: 0.03 and 0.02). Mean P1 amplitude values showed nonsignificant increase at month 6 and month 12 (p: 0.03 and 0.018). Mean baseline N1 and P1 implicit time showed a nonsignificant reduction at months 6, and 9 and a significant reduction at month 12 after Bonferroni correction (p:

Table 2 Changes in BCVA and macular thickness during the follow-up

Time	LogMAR BCVA	95% Confidence interval		*p*	Macular thickness	95% Confidence interval		*p*
		Lower limit	Upper limit			Lower limit	Upper limit	
Baseline	0.58±0.12				413.5±22.2			
Month 3	0.40±0.15	0.14	0.22	0.003	366.5±30.8	− 5.17	88.9	0.021
Month 6	0.40±0.21	0.12	0.23	0.010	344.9±23.7	13.19	120.9	0.011
Month 9	0.42±0.16	0.11	0.20	0.006	343.7±23.5	4.60	113.9	0.003
Month 12	0.37±0.21	0.17	0.24	0.005	329.5±25.3	16.43	139.7	0.005

BCVA Best corrected visual acuity

0.02, 0.02 and 0.004 for N1 implicit time; and *p*: 0.03, 0.02 and 0.01 for P1 implicit time) (Table 3).

Second Ring (5–10°): Baseline mean N1 amplitude values showed a non-significant increase over the time. Mean N1 implicit time showed a statistically non-significant reduction by month 6, and a significant reduction at months 9 and 12 (*p*: 0.01 and 0.007). Mean P1 amplitude values showed insignificant fluctuations over the time. Mean P1 implicit time values showed non-significant reductions at month 6 and month 12 (*p*: 0.03 and 0.04) (Table 4).

Third Ring (10–15°): The mean N1 amplitude values did not show statistically significant changes over the time. Mean N1 implicit time showed a statistically non-significant reduction at month 9 (*p*: 0.02) and a significant reduction at month 12 (*p*: 0.01). Mean P1 amplitude showed non-significant increases at months 3 (*p*: 0.03) and 6 (*p*: 0.02). Mean P1 implicit time values showed a non-significant reduction at month 6 (*p*: 0.02), and a significant reduction at months 9 and 12 (*p*: 0.01 and 0.007) after Bonferroni correction (Table 5).

Fourth Ring (15° Periphery): Mean N1 amplitude values showed significant increases at month 12 (*p*: 0.01). Mean N1 implicit time showed only statistically nonsignificant reduction at month 12 (*p*: 0.02) after Bonferroni correction. Mean P1 amplitude values showed a non-significant increase at month 12 (*p*: 0.02). Mean P1 implicit time values did not show significant changes over the time (Table 6).

A representative case with baseline and final mf-ERG recording together with OCT images is given in Fig. 1.

Full-field electroretinography findings

In the combined rod-cone responses, no significant changes were observed for amplitude and implicit values, but a significant increase was detected in the b amplitude value at month 12, and a significant reduction was detected in the b-wave implicit value at month 12. In the 30-Hz flicker responses, no significant change was detected for a and b amplitude and implicit values at month 12 (Table 7). A representative case with baseline and final ff-ERG recordings (combined rod cone response and cone 30-Hz flicker response) is given in Fig. 2a–d.

Correlation between mf-ERG findings and the BCVA findings

When analyzing the correlation between the baseline BCVA and the baseline N1-wave amplitude and implicit time, a negative correlation was observed between the first ring N1-wave amplitude value and the baseline BCVA. There was a negative correlation between the baseline first ring and the second ring N1-wave amplitude value and BCVA at month 12.

Analysis of the correlation between baseline BCVA and baseline P1 amplitude values showed a negative correlation between baseline first ring ($p < 0.004$; r: − 0.406) and the second ring ($p < 0.050$; r: − 0.281) P1-wave amplitude and BCVA acuity.

There was a negative correlation between the baseline first ring, the second ring P1-wave amplitude value and the BCVA at month 12.

Table 3 Changes in the central (0–5 degree) responses N1-wave amplitude implicit and P1-wave amplitude implicit values over 12 months in patients with diabetic macular edema treated with intravitreal ranibizumab injections

Central mf-ERG responses (0–5 degree) of N1 and P1 amplitudes and implicit times															
Time	N1 Amplitude (nv/deg2)	95% CI		*p*	N1 Implicit (ms)	95% CI		*p*	P1 Amplitude (nv/deg2)	95% CI		*p*	P1 Implicit (ms)	95% CI		*p*
		LL	UL			LL	UL			LL	UL			LL	UL	
Baseline	− 20.30±24.2				29.10±0.39				39.88±31.7				49.18±0.47			
Month 3	− 21.70±20.1	− 49.8	91.8	0.12	28.02±0.41	− 0.14	2.23	0.07	39,67±30.0	− 89.6	96.0	0.088	48.10±0.43	− 0.21	2.32	0.069
Month 6	− 21.61±21.5	− 25.0	92.7	0.09	27.60±0.39	− 0.15	2.60	0.02	42.61±29.5	− 91.4	47.6	0.031	46,19±0.43	− 0.72	3.26	0.035
Month 9	− 22.06±22.1	− 66.6	59.2	0.03	26.75±0.41	1.09	2.53	0.02	37.63±32.4	− 53.1	71.5	0.073	44.13±0.22	3.63	6.46	0.021
Month 12	− 23.60±21.0	− 26.3	90.5	0.02	26.61±0.45	0.23	2.88	0.004	44.72±33.7	− 69.1	81.3	0.018	44.09±0.37	0.83	3.35	0.013

CI Confidence interval, *LL* Lower limit, *UL* Upper limit

Table 4 Changes in the ring-2 (5–10 degree) N1-wave amplitude implicit time and P1-wave amplitude implicit time values over 12 months in patients with diabetic macular edema treated with intravitreal ranibizumab injections

mf-ERG responses of 5-10 degree (Ring 2) N1 and P1 amplitudes and Implicit Times															
Time	N1 Amplitude (nv/deg2)	95% CI		*p*	N1 Implicit (ms)	95% CI		*p*	P1 Amplitude (nv/deg2)	95% CI		p	P1 Implicit (ms)	95% CI		p
		LL	UL			LL	UL			LL	UL			LL	UL	
Initial	− 22.90±20.8				28.50±0.33				42.45±23.1				46.70±0.66			
Month 3	− 22.97±19.0	− 20.1	101.	0.12	27.90±0.41	− 0.37	1.67	0.08	44.00±26.1	− 93.8	2.2	0.076	47.30±0.63	− 1.32	2.17	0.080
Month 6	− 23.37±18.9	− 48.8	62.3	0.09	27.40±0.33	− 0.14	2.13	0.09	45.01±25.7	− 99.1	53.4	0.0102	45.80±0.61	0.06	3.75	0.036
Month 9	− 23.31±17.3	− 49.5	61,3	0.07	26.26±0.38	1.08	3.48	0.01	43.81±25.9	− 4.83	5.6	0.087	46.85±0.63	3.04	6.69	0.094
Month 12	− 23.60±21.2	− 50.6	73.3	0.07	26.20±0.33	0.24	2.43	0.007	43.90±23.5	− 97.8	3.8	0.090	45,12±0.72	0.49	4.71	0.041

CI Confidence interval, *LL* Lower limit, *UL* Upper limit

Table 5 Changes in the ring-3 (10–15 degree) N1-wave amplitude implicit time and P1-wave amplitude implicit time values over 12 months in patients with diabetic macular edema treated with intravitreal ranibizumab injections

10–15 degree (ring 3) N1 and P1 amplitudes and implicit times															
Time	N1 Amplitude (nv/deg2)	%95 CI		*p*	N1 Implicit (ms)	%95 CI		*p*	P1 Amplitude (nv/deg2)	%95 CI		*p*	P1 Implicit (ms)	%95 CI		*p*
		LL	UL			LL	UL			LL	UL			LL	UL	
Baseline	− 27.10±20.3				28.35±0.43				47.19±2.31				50.40±0.48			
Month 3	− 26.91±18.5	− 58.4	60.6	0.09	27.66±0.34	− 0.29	1.67	0.074	50.50±2.34	− 8.76	3.10	0.028	50.02±0.58	46.8	49.1	0.092
Month 6	− 25.45±18.5	− 79.2	29.0	0.09	27.21±0.36	− 0.12	2.40	0.092	50.72±1.76	− 8.91	2.96	0.017	48.70±0.36	46.0	47.4	0.020
Month 9	− 28.63±18.9	− 102.	5.78	0.07	26.40±0.29	0.87	2.91	0.019	43.98±1.47	− 3.74	8.93	0.077	45.60±0.24	43.1	44.1	0.011
Month 12	− 25.50±18.5	− 78.0	32.7	0.10	26.25±0.34	0.33	2.67	0.014	48.10±1.35	− 7.61	5.52	0.090	44.80±0.34	46.1	47.5	0.007

CI Confidence interval, *LL* Lower limit, *UL* Upper limit

Table 6 Changes in ring-4 (15 degree periphery) N1-wave amplitude implicit time and P1-wave amplitude implicit time values over 12 months in patients with diabetic macular edema treated with intravitreal ranibizumab injections

mf-ERG responses of 15 degree periphery (Ring 4) N1 and P1 amplitudes and Implicit Times															
Time	N1 Amplitude (nv/deg2)	95% CI		*p*	N1 Implicit (ms)	95% CI		*p*	P1 Amplitude (nv/deg2)	95% CI		*p*	P1 Implicit (ms)	95% CI		p
		LL	UL			LL	UL			LL	UL			LL	UL	
Baseline	− 28.82±18.8				28.72±0.29				53.65±2.57				48.73±0.67			
Month 3	− 30.07±23.1	− 26.6	83.7	0.081	27.76±0.39	0.11	1.81	0.910	55.78±2.88	50.0	61.4	0.13	47.95±0.61	46.7	49.1	0.099
Month 6	− 27.40±17.4	− 87.2	47.8	0.092	27.33±0.36	0.22	2.55	0.110	52.49±13.51	48.9	56.0	0.082	46.65±0.39	45.8	47.4	0.13
Month 9	− 27.50±19.4	− 69.8	32.3	0.078	26.12±0.30	1.53	3.67	0.099	51.77±1.77	49.1	47.9	0.074	48.63±0.28	43.1	44.2	0.084
Month 12	− 31.83±16.9	4.08	10.1	0.014	26.01±0.31	0.70	3.13	0.019	59.83±10.39	46.7	52.1	0.018	47.20±0.29	46.6	47.8	0.081

CI Confidence interval, *LL* Lower limit, *UL* Upper limit

Table 7 Summary of electrophysiologic data from full-field electroretinograms for patients treated with ranibizumab for diabetic macular edema

Data type		Baseline		Month 12		*P*
		Amplitude (micronVolt)	Implicit time (ms)	Amplitude (micronVolt)	Implicit time (ms)	
Combined rod-cone	a-wave	-48.26	31.63	-53.76	34.77	0.02 (A)/0.50 (IT)
	b-wave	243.7	60.40	265.0	45.42	0.072 (A)/0.000 (IT)
30 Hz flicker	a-wave	-41.34	36.62	-32.64	36.35	0.27 (A)/0.77 (IT)
	b-wave	34.08	45.21	44.04	44.85	0.006 (A)/0.69 (IT)

A Amplitude, *IT* Implicit time

Correlation between mf-ERG findings and the central macular thickness

A positive correlation was observed between the baseline CMT and the second ring ($p < 0.015$; r: − 0.333), the third ring, fourth ring N1-wave implicit time at baseline. A positive correlation was observed between the baseline CMT and the first ring ($p < 0.005$; r: 0.356) and second ring N1-wave implicit time at month 12.

A positive correlation was observed between the baseline CMT and the first ring, the second ring the third ring implicit time at baseline.

*Correlation analysis between the full-field ERG findings and the visual acuity showed n*o meaningful correlation at any time points was observed.

Correlation between the full-field ERG findings and the central macular thickness showed a negative correlation between the combined response b-wave amplitude and the baseline CMT. There was also no correlation between the injection number and any measurement of mfERG or ff-ERG at any time point. All meaningful correlations with significance values are shown in Table 8. The regression analysis showed that the only predictive factor for final visual results was baseline BCVA (B: 1.02, *p*: 0.00).

Discussion

In our study, we observed statistically significant improvements in visual acuity and macular thickness starting from the third month after initiation of ranibizumab treatment. This improvement was achieved with 6.19 intravitreal ranibizumab injections on average. However, multifocal ERG recordings generally improved at month 9 and 12. So, we found that macular electrophysiologic improvement occurs on the long term after anti-VEGF treatment. We also found that global retinal functional status does not worse, but some improvement can occur after anti-VEGF treatment over one year period.

As a neurophysiological improvement, increases in the amplitude values and reductions in the implicit time were recorded. While some improvements were observed for the third and fourth rings, they were most common for the first and the second rings that reflect the fovea and the parafovea values. Because N1-wave includes contributions from the same cells that contribute to the a-wave of the light-adapted, full-field ERG and that P1 and N2 include contributions from the cells contributing to the light-adapted b-wave and oscillatory potentials [34], improvement in mf-ERG recordings at months 9 and 12 basically implies a neurophysiological enhancement of photoreceptors in the long term after intravitreal ranibizumab treatment.

Reduction in the oscillatory potential amplitude, delay in wave formation time, decrease in photopic–scotopic a- and b-wave amplitudes, and delay in cone response on flicker recordings have been detected along with the presence and progression of retinopathy [30, 31, 35]. Thus, the change and the progress in retinal neuropathy responses resulting from retinopathy can be recorded by using ff-ERG. Holm et al. [31] investigated the effects of intravitreal ranibizumab treatment on peripheral retinal health using ff-ERG recordings, the reduction in the implicit time relative to baseline was found significant on 30-Hz flicker recordings. No significant difference was detected between the rod amplitudes. Comyn et al. [30]

Table 8 Correlations between morpho-functional results

Parameter	Correlation	*p*	r
Baseline BCVA*	Baseline mf-ERG N1-wave amplitude central area	0.019	− 0.37
	Baseline mf-ERG P1-wave amplitude central area	0.003	− 0.41
	Baseline mf-ERG P1-wave amplitude 5–10 degree	0.031	− 0.28
Month 12 BCVA	Baseline mf-ERG N1-wave amplitude central area	0.026	− 0.34
	Baseline mf-ERG N1-wave amplitude 5–10 degree	0.035	− 0.27
	Baseline mf-ERG P1-wave amplitude central area	0.004	− 0.38
	Baseline mf-ERG P1-wave amplitude 5–10 degree	0.050	− 0.26
	Baseline mf-ERG N1 implicit time 5–10 degree	0.031	0.32
	Baseline mf-ERG N1-wave implicit time 10–15 degree	0.016	0.36
Baseline CMT	Baseline mf-ERG N1-wave implicit time 5–10 degree	0.015	− 0.33
	Baseline mf-ERG N1-wave implicit time 10–15 degree	0.004	− 0.37
	Baseline mf-ERG N1-wave implicit time 15 degree	0.008	− 0.35
	Baseline mf-ERG P1-wave implicit time central area	0.025	− 0.30
	Baseline mf-ERG P1-wave implicit time 5–10 degree	0.020	− 0.31
	Baseline mf-ERG P1-wave implicit time 10–15 degree	0.015	− 0.33
	Month 12 mf-ERG N1-wave implicit time central	0.005	− 0.36
	Month 12 mf-ERG N1-wave implicit time 5–10 degree	0.005	− 0.38
HbA1C	Month 12, mf-ERG p1-wave implicit time 5–10 degree	0.03	0.33
	Month 12, mf-ERG p1-wave implicit time 10–15 degree	0.04	0.31
	Baseline ff-ERG b-wave amplitude	0.03	− 0.33
	Month 12 ff-ERG b-wave amplitude	0.03	− 0.32
Injection number	Baseline LDL level	0.013	0.69
Injection number	Baseline CMT	0.014	0.36
VLDL	Baseline mf-ERG P1-wave implicit time central area	0.044	0.59
	Month 12, mf-ERG P1-wave implicit time 5–10 degree	0.002	0.81
	Baseline ff-ERG 30 Hz Flicker b-wave amplitude	0.022	− 0.65
TRG	Baseline mf-ERG N1-wave implicit time 10–15 degree	0.035	0.64
	Month 12, mf-ERG P1-wave implicit time 5–10 degree	0.04	0.63
	Month 12, mf-ERG P1-wave implicit time 5–10 degree	0.034	0.64
	Month 12, mf-ERG N1-wave implicit time 10–15 degree	0.03	0.65

**BCVA* represents converted LogMAR units for statistical evaluation

evaluated the peripheral retinal function using ff-ERG at week 48 in eyes with DME treated with intravitreal ranibizumab. Rod system function was assessed by the dark-adapted ERGs. There was no change identified in the dim-flash ERG B-wave in either group over 48 weeks. The mean A-wave and B-wave amplitude in the scotopic brighter-flash ERG decreased in the ranibizumab group with no change in peak time, but the difference was not significant. They stated that, although a mild loss of function cannot be excluded, there was no evidence of generalized dysfunction. In consistence with this study, ff-ERG recordings in our study showed that ranibizumab treatment had no unfavorable effects on the peripheral retina, and in contrast, there was an improvement in some parameters at month 12. No toxicity associated with anti-VEGF effect on retinal cells has been reported. On the contrary, electroretinographic improvement has been attributed to the improvement in retinopathy level secondary to the anti-VEGF efficacy [36, 37]. Our study is in line with previous studies.

Multifocal ERG may objectively reflect macular neuronal function in eyes with DME. Delay in mf-ERG responses and reduction in amplitudes have been reported in diabetic macular edema [38]. In type-2 diabetic patients, mf-ERG recordings showed significantly lower N1- and P1-wave amplitude and prolonged implicit times relative to cases with type 1

diabetes. This result is consistent with previous studies [44]. However, although adult type 2 diabetes patients are the main patient group, mixing two types may have an effect on the results of mf-ERG amplitudes in our study. ERG findings in diabetic patients support the concept that functional loss may occur before the manifestation of retinopathy findings [39]. mf-ERG findings have been reported to be correlated with perimetry findings in diabetic eyes and the amplitude values showed a better correlation than the implicit time [40]. In a similar previous study, twenty patients underwent mf-ERG, ff-ERG, OCT, rapid blood sugar measurement, and HbA1c measurement four weeks after the initial injection and four weeks after the third injection. While implicit times significantly decreased following the first injection in the third ring, an increase was observed similarly in the amplitudes; however, this increase was found significant only in the central ring. Following the third injection, the amplitude values and implicit times returned to levels close to the baseline [41]. The authors reported that the neuroretinal improvement could take time. In another study with a 6-month follow-up, no improvement was observed in PERG and mf-ERG in eyes with DME treated with intravitreal ranibizumab injections. Reduction in the macular edema at month 3 was attributed to the improvement in synaptic connections; however, this could not be maintained at month 6 [32]. Our 3- and 6-month results are consistent with the previous short term studies. Comyn et al. [30] investigated the mf-ERG responses at 12, 24 and 48 weeks in patients with DME, in whom they planned laser or intravitreal ranibizumab treatment. They reported that the baseline central macular function was 70% lower relative to the normal data of their laboratory. One-third of the patients treated with ranibizumab were detected to have mild to moderate improvement in central macular responses; however, nearly half of the patients had no significant changes in central macula responses at 12, 24, and 48 weeks. 14% of the patients treated with ranibizumab had a reduction in mf-ERG responses at 48 weeks.

Strong correlation between visual acuity and both N1- and P1-wave amplitude values was noticeable. In our study, there was a positive correlation between baseline central macular thickness and baseline N1-wave implicit time in the second and third and fourth rings, and to P1-wave implicit time in the first, second, and third rings. In cases with shorter baseline P1-wave implicit times, post-treatment anatomic improvement was better. This finding may indicate that anatomic improvement was better or more rapid in patients with preserved middle retinal layers and particularly bipolar and amacrine cells. We do not know yet whether better baseline N1- and P1-wave implicit values can be considered a prognostic indicator of anatomic improvement. Similarly, the baseline central macular thickness and 12-month N1-wave implicit time revealed a positive correlation in the first and second rings. This finding may indicate that thicker baseline macula enables longer final N1-wave implicit time, and thus, a functional improvement in photoreceptors will take longer.

Inner and outer plexiform layers mainly affected by DME and Müller cells may be disorganized with adjacent neuronal cells, but inner and outer nuclear layers can also be involved in time, therefore, horizontal or bipolar cells and amacrine cells as well as synaptic extensions of photoreceptor can be affected [38, 42]. In our study, the fact that morphological improvement starts from month 3 while electrophysiological improvement starts from month 6 should be of significance. Thus, retinal cellular functional restoration takes longer time than anatomical restoration. Cellular functional recovery in the middle retinal layer and RPE-photoreceptor junction may occur slowly. Thus, physiological restoration in retinal neuronal cells and recovery of synaptic connections are possible within a specified period if the macula is protected against recurrences of edema. Our results may suggest that the visual function secondary to global macular health would be better as the treatment continues or macula remains dry. The inconsistency between anatomic and electrophysiological improvement at early period can be explained by impairment of the sensitive intra-retinal mechanisms due to edema. One of the most interesting findings was the statistically significant improvement in P1- and N1-wave values at month 12, which was evident in almost all rings. Studies with a follow-up period of more than 12 months may reveal whether the change in electrophysiological data is transient or permanent.

Our study has several limitations. ERG measurements can show high test–retest variability [33, 43]. One limitation of our study is that we did not evaluate the test–retest variability as we did not perform the

tests twice in one session. We did not classify DME types. Different DME types may give different responses to Anti-VEGF agents in terms of thickness and function [18]. In addition, we did not recorded oscillatory potentials in performing ff-ERG study, although it is valuable to assess global retinal health in eyes with diabetic retinopathy. Another limitation may be possible metabolic fluctuations over the study period, because we did not monitor any metabolic parameters such as HbA1C or blood sugar levels.

In conclusion, we observed statistically significant improvements in BCVA and macular thickness values starting from the month 3 with intravitreal ranibizumab treatment, and there were improvements in mf-ERG measurements, beginning from month 6 and continuing at months 9 and 12. Full-field ERG showed some improvement in the peripheral neuroretinal cells at month 12. The high N1-P1-wave amplitude values at treatment onset may indicate a good prognosis. Patients with lower macular thickness were observed to have better electrophysiological improvements, particularly in the macula center. Macular thickness was found to be strongly correlated with implicit time, and the visual results were strongly correlated with amplitude time. However, long-term studies are needed to support our results.

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Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration 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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