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ORIGINAL PAPER

Multifocal electroretinogram in diabetic macular edema and its correlation with different optical coherence tomography features

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Abstract

Purpose To evaluate multifocal electroretinogram (mfERG) parameters in eyes with diabetic macular edema (DME) and its correlation with vision and optical coherence tomography (OCT) features.

Methods Fifty-four eyes of 27 subjects with DME due to nonproliferative diabetic retinopathy were evaluated. MfERG responses were measured in three concentric rings. Macular thickness was measured by OCT in each segment of the three concentric rings, and mfERG rings were superimposed on the macular thickness map. The correlation between macular thickness in specific points of the thickness map and changes of the mfERG parameters in the corresponding points of the mfERG field map was evaluated and the relationship between the OCT and mfERG changes and changes of best-corrected visual acuity (BCVA) was investigated. The central foveal B-scans of SD-OCT were used to evaluate any correlation between the external limiting membrane (ELM) status, ellipsoid zone (EZ) status, presence of cysts or disorganization of retinal inner layers (DRIL), and mfERG parameters at the central corresponding area.

Results The mean of BCVA was 0.5 ± 0.3 in logMAR, and the central macular thickness was 392.6 ± 123.4 microns. The central ring P1 and N2 amplitudes had a significant correlation with BCVA in univariate and multivariate analyses ($P = 0.001$ for both, $r = -0.346$ and $r = -0.646$, respectively). There was a significant correlation between retinal thickness and the N1 amplitude in the central ring ($P = 0.02$, $r = -0.343$). Outer retinal layer disruption (ELM and EZ) correlated with prolonged P1 implicit time at the corresponding location ($P = 0.005$, $r = 0.068$). The presence of the DRIL was associated with reduced P1 and N2 amplitudes ($P = 0.037$, $r = -0.284$ and $P = 0.019$, $r = -0.562$, respectively). A significant correlation was also found between the presence of cysts and a lower central P1 amplitude ($P = 0.033$, $r = -0.376$).

Conclusion In diabetic patients, discrete changes of some parameters in the central ring of the mfERG field map (e.g., P1 and N2 amplitudes) have a significant correlation with both structural OCT abnormalities in the corresponding points of the thickness map (like DRIL, intraretinal cyst and ELM/EZ disruption) and BCVA. Predictive models such as those described in this report may make it possible to identify the relationship between specific anatomical and functional characteristics in diabetic retinopathy.

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Introduction

Diabetic macular edema (DME) is the main cause of visual loss in diabetic patients [1]. It has been reported that 20% and 14% of subjects with type 1 and type 2 diabetes mellitus (DM) develop macular edema, respectively [2]. Diabetic retinopathy (DR) affects both neurologic and vascular parts of the retina. It has been hypothesized that a blood–retinal barrier dysfunction secondary to the effects of glucose on the vascular walls may lead to retinal microvascular abnormalities. Otherwise, neuropathy occurs after conduction delay in neural cells due to the influence of hyperglycemia on neurotransmitters [3]. Pilot investigations have shown that neuropathy may precede or accompany vasculopathy in patients with diabetic retinopathy. Subclinical "neuro-retinopathy" has been also reported in diabetic patients with no diabetic retinopathy [4, 5].

Multifocal electroretinogram (mfERG) was developed by Sutter and Tran in 1992 to evaluate the retinal electrophysiological activity and provide a topographic map [6]. It can record focal ERG responses from various loci simultaneously in the central 40° to 50° of the retina (20°–25° radius from the fixation point) [7]. The mfERG is taken under light adaptation and obtains an electrophysiological response from retinal cone cells [8]. The implicit time of the mfERG waves is significantly longer in diabetic cases without obvious retinopathy. In patients with diabetic retinopathy, the implicit time becomes even longer with an increase in the severity of retinopathy [9]. According to the results of multivariate analysis, the mfERG implicit time can be used as a predictor of DR development in patients with no obvious retinopathy [10].

Optical coherence tomography (OCT) is a noninvasive imaging modality that provides in vivo structural information as well as objective quantitative measurements of the retinal morphological changes [11]. OCT permits precise assessment of the macular structural changes; however, this structural measure correlates only modestly with visual acuity [6]. Newer versions of OCT can be used to evaluate the retinal structure on a layer-by-layer basis. Recent studies demonstrated correlations between visual acuity (VA) and specific retinal OCT, such as impairment of the external limiting membrane (ELM), ellipsoid zone (EZ) and interdigitation line [12]. The presence of disorganization of retinal inner layers (DRIL) in the central retina correlates with the severity of retinopathy and poor visual acuity in diabetic patients [13].

Few studies have evaluated the combined use of OCT and mfERG to investigate the correlation between macular structural and functional changes in patients with DME [3, 6–8]. This study was conducted to evaluate the changes of multifocal electroretinogram (mfERG) parameters in eyes with DME and its correlation with visual acuity and OCT features.

Methods

This cross-sectional observational study was conducted from August 2017 to March 2018. Institutional ethical clearance was obtained prior to commencement of the study. Informed consent was taken from all participants. The study was conducted in accordance with the Declaration of Helsinki and its later amendments.

Data were collected from all treatment naive DME patients who were 40–70 years old and were diagnosed by a group of retina specialists in the Retina Clinic of Farabi Eye Hospital. All patients underwent (manifest) refraction, best-corrected visual acuity (BCVA) measurement, slit lamp biomicroscopy, and OCT imaging. The patients were included in the study if they did not have type 1 DM, proliferative diabetic retinopathy, uncontrolled glaucoma or previous ocular surgery, significant media opacity, poor central or unsteady fixation, poor cooperation, poor controlled DM (HbA1c $> 8\%$) and any other ocular or systemic disorders affecting retinal function. The data collectors explained the study protocol and objective, imaging modalities (fluorescein angiography and mfERG), and probable disadvantages and advantages to the participants. After providing informed consent, the patients underwent fluorescein angiography (FA) and those with any significant enlargement of the foveal avascular zone were excluded to rule out macular ischemia as a cause of decreased visual

acuity. Finally, mfERG responses were recorded from standard points of the retina by an expert retinal electrophysiologist.

Optical coherence tomography

OCT was performed using a spectral domain device (Spectralis HRA-OCT, version 5.3.3.0; Heidelberg Engineering, Heidelberg, Germany). A raster imaging protocol consisting of 31 horizontal scans was obtained, which covered a 7×9 mm^2 area centered on the fovea. The retinal thickness of the central 1.0 mm was defined as the central macular thickness (CMT), and patients with a CMT more than 300 μm were included in the study. The retinal thickness of the central 3.0 mm was calculated in each of the 4 quadrant areas in Early Treatment Diabetic Retinopathy Study (ETDRS) grid, which was provided automatically by the macular cube scan.

The B-scan cuts, which passed through the deepest part of the foveal depression, were identified and defined as the central foveal B-scan. The presence of ELM, EZ, interdigitation line disruption, cyst and DRIL in the central foveal B-scan was determined. When the central foveal scan was not identifiable, the scan offset by 5° from the horizontal line was used as the preferred scan. The type of DME in each eye was classified according to the presence or absence of cystic changes. The presence of subretinal fluid was determined in each case.

Based on the EZ and ELM status, all eyes were divided into two groups: intact and disrupted. The "intact group" was defined as eyes with a regular continuity of the hyperreflective lines corresponding to the ellipsoid zone or external limiting membrane, and the "disrupted group" was characterized by the presence of any discontinuity in these lines.

The DRIL was defined as the presence of a locus on the OCT images where the margins between the inner retinal layers could not be separately identified. Based on the presence of DRIL, the eyes were also categorized into two groups: DRIL positive and DRIL negative.

Multifocal ERG (mfERG)

The Metrovision system (Vision Monitor, Perenchies, France) was used for mfERG according to the International Society for Clinical Electrophysiology of Vision (ISCEV) guidelines. The stimulation matrix consisted of a hexagonal mfERG stimulus array with 61 elements displayed on a cathode ray tube monitor at a frame rate of 120 Hz. The highest available frame rate frequency was selected to provide better temporal resolution. The viewing distance was set at 33 cm, which corresponded to a stimulated field of ± 30° horizontally and ± 24° vertically. A resolution of 1024×768 corresponds to 3.6 arc minutes at the default viewing distance. According to the eccentricities, the amplitudes and latencies were evaluated in 3 concentric retinal ring regions (central, middle, and outer ring). The proper focus and location of the stimulation image were monitored with an infrared fundus video system on a computer screen. Corneal ERG-Jet contact lens electrodes were used as the active recording electrode of mfERG, while the pupils were fully dilated. The fellow eye was occluded, and the eye position was monitored on a computer screen. The patients were asked to fixate on the central target. The recording procedure was repeated if there were false potentials from eye blinking or movements.

The components of the first-order kernel (FOK) of N1, N2, and P1 in three concentric rings centered on the fovea were documented (i.e., 0°, 2°, 5°, and 10°). The N1 amplitude was measured from the baseline to the N1 trough, the P1 amplitude was measured from the N1 trough to the P1 peak, and the N2 amplitude was measured from the P1 peak to the N2 trough. The N1, N2, and P1 latencies were measured from the time of presenting the stimuli. The responses were analyzed according to ring averages in all four quadrants.

As presented in Figs. 1 and 2, the mfERG hexagonal patterns were superimposed over OCT patterns to evaluate any correlation between macular thickness and mfERG parameters in each region. For each of the N1, N2, and P1 amplitudes and latency measurements, a separate hexagonal map with the ETDRS grid was generated for better superimposition on the OCT thickness maps with a standard ETDRS grid. We have checked the centration of both modalities according to the fovea location before superimposing corresponding images. All the superimposed images were confirmed by 3 retina specialists separately.

For each area of sectors, the mfERG responses including the N1 amplitude and latency, P1 amplitude and latency, and N2 amplitude and latency were averaged for analysis. Only the central foveal B-scan

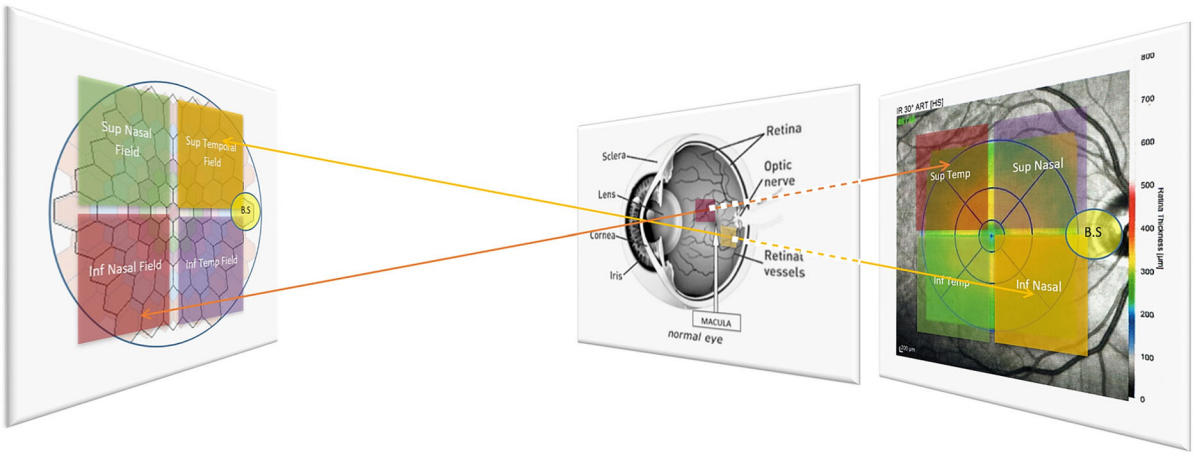

Fig. 1 A diagram shows structural anatomic location of normal retinal areas and their correlation with multifocal ERG field map in the right eye. An inferonasal retinal area corresponding to the fovea correlates with superotemporal field of multifocal ERG (orange rectangles), and a superotemporal retinal area corresponding to the fovea correlates with inferonasal field of multifocal ERG (red rectangles). *BS* bind spot

was used to evaluate any correlation between OCT biomarkers (like ELM status, EZ status, interdigitation line status and presence of the cyst or DRIL) and mfERG parameters at the central corresponding area.

Statistical methods

The Kolmogorov–Smirnov test was applied to check the assumption of normality. Correlation, univariate, and multivariate regression analyses were performed on VA, CMT, and amplitude and latency of N1, N2, and P1 waves. The Pearson's coefficient was used to evaluate the correlations. OCT and mfERG were compared in terms of the presence of DRIL, ellipsoid zone, interdigitation line, and ELM integrity using the Kruskal–Wallis test, and post hoc analyses were conducted with the Mann–Whitney U test and a Bonferroni correction. Multivariate multiple regression was used to evaluate the effect of each potential factor. The SPSS software version 22.0 (IBM Corp., Armonk, NY) was used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

Demographic features

Thirty patients were enrolled in the study. All the patients had mild to severe nonproliferative diabetic retinopathy (NPDR) based on a funduscopic examination. Three patients were excluded due to significant enlargement of the FAZ area in FA. Fifty-four eyes of 27 treatment naive patients (15 male and 12 female) with center-involved DME due to nonproliferative diabetic retinopathy underwent mfERG. The mean HbA1c of the patients enrolled in this study was 6.88 ± 1.28 mg/dl. The mean age of the patients was 59.3 ± 7.3 years, ranging from 46 to 71 years. The mean BCVA was 0.5 ± 0.3 in logMAR and the mean central macular thickness was 392.6 ± 123.4 µm.

mfERG parameters and BCVA

The correlations between the visual acuity and the amplitude or latency of mfERG waves in all three circles were evaluated using the Pearson's correlation analysis. The correlation between BCVA and the amplitude of P1 and N2 waves was significant in the central ring area ($P = 0.001$ for both). The association between BCVA and other mfERG parameters in the central macular area was not significant except for a significant inverse association between BCVA and central N1 latency ($P = 0.012$).

On the other hand, multivariate regression analysis showed a significant association between BCVA as a dependent variable and the central P1 and N2 amplitude ($P = 0.001$); therefore, both P1 and N2 amplitudes correlated significantly with BCVA in univariate and multivariate analysis. There was no significant

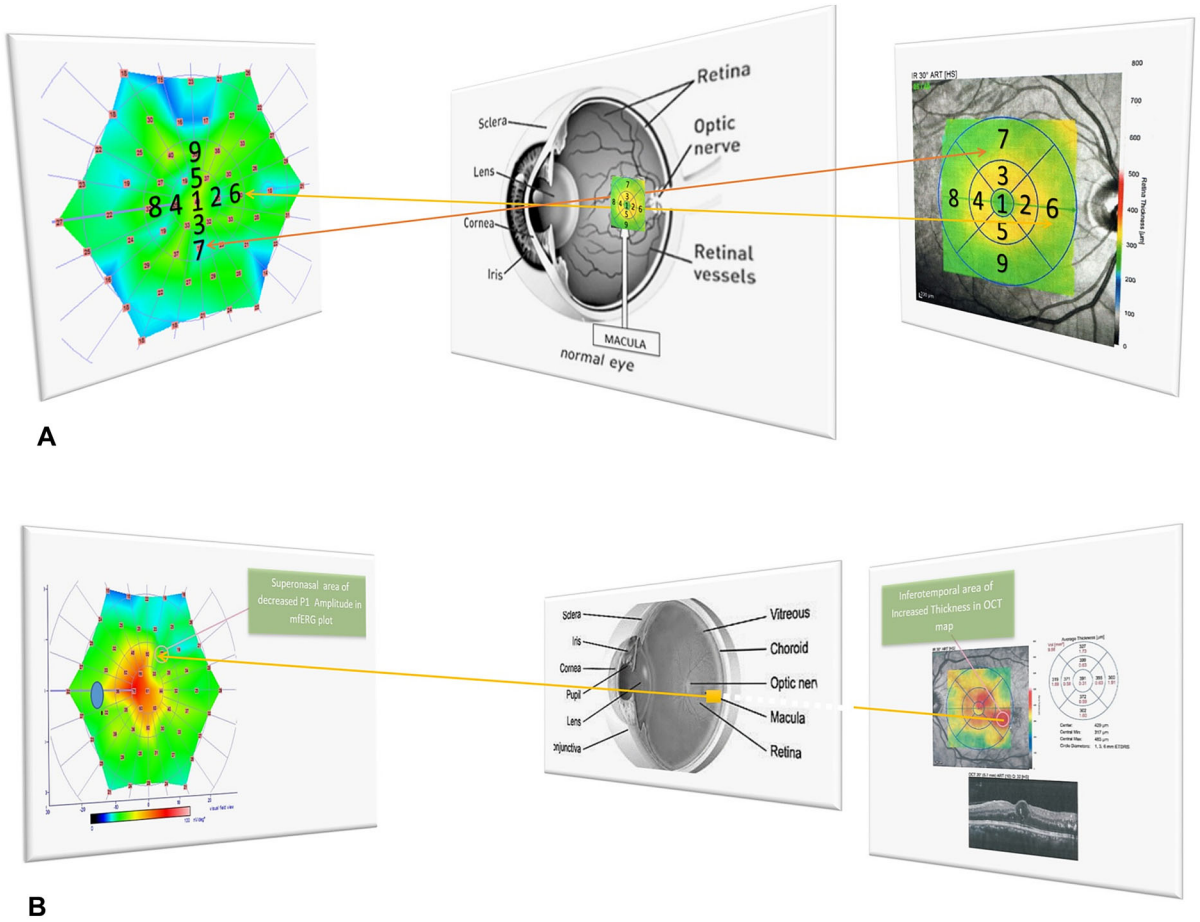

Fig. 2 **a** Diagram showing the correlation between ETDRS regions of the normal macula in OCT and multifocal ERG field map in the right eye. Nasal region in macular area (area number 6) corresponds to temporal field and superior region in macular area (area number 7) corresponds to inferior field in multifocal ERG field map. **b** A diagram linking structural anatomic location of increased retinal thickness area with multifocal ERG field in the left eye of a patient with diabetic macular edema. An inferotemporal retinal area corresponding to the fovea that shows increased thickness in OCT map correlates with superonasal field of multifocal ERG showing decreased P1 amplitude in that area (Orange Arrow)

correlation between the amplitude and implicit time of mfERG parameters for the 2–5° and 5–10° ring and BCVA (Table 1).

Structural OCT and BCVA

There was no statistically significant correlation between BCVA and central macular thickness (P = 0.065).

Structural OCT and mfERG parameters

Retinal thickness had a significant correlation with N1 amplitude in the central ring (P = 0.02). There was no statistically significant correlation between CMT and mfERG parameters (neither amplitude nor latency) in other regions (Table 2).

Based on the ellipsoid zone (EZ) status, 38 eyes (70.3%) had intact EZ's and 16 eyes (29.7%) had disrupted EZ's. The external limiting membrane (ELM) was intact in 40 eyes (74%) and disrupted in 14 eyes (26%). Disorganization of retinal inner layers (DRIL) was found in 8 of 54 eyes (14.8%). Thirty-seven eyes (68.5%) had cystic changes versus 17 eyes (31.5%) that had no cystic changes. Subretinal fluid was documented in 5 eyes.

A multiple linear regression model was also conducted to estimate the visual acuity based on

Table 1 Correlations between BCVA and parameters of mfERG including amplitude and latency

	N1 amplitude		N1 implicit time		N2 amplitude		N2 implicit time		P1 amplitude		P1 implicit time	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Central	− .304	.148	.487	**.012**	− .346	**.001**	.014	.951	− .646	**.001**	.420	.114
Inferior1[a]	− .143	.495	.367	.071	− .172	.410	.357	.080	− .171	.412	.422	.718
Superior1[a]	− .032	.870	.464	.067	− .176	.786	.068	.751	− .032	.740	.064	.836
Temporal1[a]	− .295	.172	.345	.095	− .052	.886	.014	.952	− .124	.573	.057	.791
Nasal1[a]	− .282	.179	.254	.260	− .271	.191	.096	.648	.335	.192	.377	.064
Inferior2[b]	− .188	.388	.202	.333	− .139	.519	.108	.608	− .276	.152	.139	.506
Superior2[b]	− .232	.252	.149	.478	− .075	.728	.229	.270	− .193	.357	.047	.828
Temporal2[b]	− .203	.334	.016	.943	− .321	.120	.184	.390	− .521	.057	.294	.164
Nasal2[b]	− .305	.150	.123	.576	− .086	.971	.014	.939	− .217	.302	.308	.143

Bold values indicate statistically significant with $p < 0.05$

[a]first ring (2–5°)

[b]second ring (5–10°)

Table 2 Correlations between central macular thickness and parameters of mfERG including amplitude and latency

	N1 amplitude		N1 implicit time		N2 amplitude		N2 implicit time		P1 amplitude		P1 implicit time	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Central TH	− .343	**.021**	.041	.789	− .146	.337	− .011	.945	− .113	.460	− .076	.618
Inferior1[a] TH	.038	.797	− .119	.417	− .384	.066	− .191	.188	− .176	.225	.022	.880
Superioe1[a] TH	.107	.470	.164	.265	− .142	.336	.127	.391	− .049	.740	.174	.236
Temporal1[a] TH	− .169	.262	.101	.505	− .082	.586	− .072	.632	− .044	.773	.014	.925
Nasal1[a] TH	.022	.878	.085	.560	− .071	.623	− .004	.979	.057	.692	.027	.852
Inferior 2[b] TH	− .129	.371	.133	.358	− .158	.274	.208	.148	− .170	.239	.143	.320
Superior 2[b] TH	− .112	.463	− .022	.884	− .113	.458	.075	.623	− .034	.823	.027	.858
Temporal 2[b] TH	.083	.571	.112	.443	.161	.270	− .118	.419	.274	.057	− .061	.679
Nasal 2[b] TH	.035	.814	.077	.602	− .086	.563	.114	.439	− .063	.672	.037	.802

Bold values indicate statistically significant with $p < 0.05$

TH thickness

[a]first ring of ETDRS chart in OCT

[b]second ring of ETDRS chart in OCT

functional and structural findings. Regression analysis showed that the central P1 and N2 amplitude ($P = 0.001$), N1 latency ($P = 0.001$), foveal EZ status ($P = 0.013$), foveal ELM status ($P = 0.015$), and the presence of DRIL ($P = 0.034$)\ correlated with BCVA.

Eyes with cystic changes had a higher central macular thickness ($P = 0.035$) and more EZ/ELM disruption ($P = 0.015$ and 0.004, respectively). By contrast, eyes without cystic changes had a lower CMT ($P = 0.001$) and better EZ/ELM status ($P = 0001$ and 0.001). Outer retinal layer disruption (ELM and EZ) had a significant correlation with a prolonged P1 implicit time at a corresponding location ($P = 0.005$), but there was no significant correlation between EZ/ELM disruption and the amplitude of the

waves. Disorganization of retinal inner layers (DRIL) was associated with a reduced P1 and N2 amplitude at corresponding locations ($P = 0.037$ and $P = 0.019$, respectively). The presence of cysts had a significant correlation with a lower central P1 amplitude ($P = 0.033$).

The presence of subretinal fluid in the macula was significantly associated with a longer implicit time of the N2 and N1 waves in the inferior and superior parts of the first ring ($P = 045$ and 0.039, respectively). The amplitudes of all waves in patients with subretinal fluid were lower compared to patients without subretinal fluid, but the differences were not significant.

Based on subgroup analysis, in patients with intact EZ/ELM and absence of DRIL, the P1 wave amplitude had a moderate correlation with BCVA ($r = -0.396$, $P = 0.04$) while BCVA had no correlation with N1 latency and N2 amplitude ($P = 0.087$).

Discussion

This study was conducted to investigate the correlation between macular OCT abnormalities (as a structural assessment), changes of mfERG parameters (as a functional assessment) and BCVA (as a subjective assessment) in patients with diabetic macular edema. The results showed that some mfERG parameters were significantly correlated with structural OCT changes and BCVA loss. In this study, similar to previous reports, CMT had no significant correlation with BCVA, [14] which may be in part explained by the fact that mfERG and BCVA evaluate the retinal function but CMT is not essentially an indicator of the retinal function [15]. These results suggest that OCT measurement alone may not be a good surrogate for visual acuity as a primary outcome in DME studies.

Based on previous studies, no statistically significant correlations were observed between central ring parameters of the mfERG and CMT [3, 6, 16]. Holm et al. found that when macular thickness exceeded 300 microns, the diminishing of amplitudes and prolongation of implicit times measured by mfERG seemed to be more pronounced [17]. The patients enrolled in our study had a mean CMT of 392 microns; therefore, it was expected to observe a change in the central mfERG parameters, while only the N1 amplitude significantly correlated with CMT. This difference can be explained by two hypotheses: 1—The stage of the disease may be a key reason for these differences as mfERG and OCT reveal the functional and structural aspects of retinal involvement in DME and all of our cases were in the nonproliferative phase; therefore, it is possible that the function of the retina was not affected significantly. However, with progression of retinopathy, both the function and structure of the retina would be severely affected and the changes of mfERG parameters and CMT would be parallel to the DME progression [9]. 2—There is evidence that N1 wave includes contributions from photoreceptors and early involvement of the N1 amplitude in our patients may suggest the potential susceptibility of the photoreceptors function to DME-related changes even in nonproliferative retinopathy in comparison to other cells like bipolars that contribute to the P1 wave of mfERG [10].

Limited data are available on the correlation of VA with the mfERG amplitude and latency [16, 18]. Goel et al. [19] recently showed that BCVA had a significant positive correlation with the P1 and N1 amplitudes and a significant negative correlation with the P1 and N1 latency in all participants. Our study also found a significant correlation between BCVA and P1 and N2 amplitudes in the central ring. However, there was no correlation between the implicit time of these waves and BCVA based on our results. The results of univariate analysis showed that the correlation between BCVA and P1 and N2 amplitudes and central N1 latency was significant while multivariate analysis showed a significant correlation only between the P1 and N2 amplitudes and BCVA. It seems that the amplitude of P1 is associated with BCVA as the most outstanding mfERG parameter in diabetic patients. A previous study suggested that the latency of the mfERG waves was more important than the amplitudes for retinal function measurement in diabetics [3]. By contrast, our study and some other recent studies emphasized the importance of amplitude. Dale et al. found that functional damage might appear on mfERG before the structural changes on OCT although OCT may show some structural changes before mfERG. Therefore, they may complete one another [18].

Previous studies showed that a decrease in or loss of visual acuity is correlated with an increase in the severity of diabetic macular edema, which may be related to the structural changes other than macular thickness like the presence of DRIL, disorganization of EZ/ELM, and cystoid changes [13, 20]. Based on

previous studies, the main OCT structural biomarkers that have an effect on the visual prognosis other than macular thickness in patients with diabetic macular edema can be categorized to inner retinal changes (like DRIL and cystic spaces on OCT) and outer retinal changes (like EZ/ELM disruptions) [13, 21–24]. None of the previous studies evaluated the effect of microstructural changes of both the inner retina and outer retina like DRIL or EZ/ELM disruption status on mfERG and visual acuity simultaneously. This study evaluated whether there was any correlation between the above OCT biomarkers and mfERG parameters. It has been hypothesized that DRIL occurs due to the loss of bipolar, horizontal, and amacrine cells within the retina. Recently, some authors suggested a new mechanism for DRIL formation. Based on this new theory, DRIL occurs when bipolar axons break due to severe edema that overcomes the elasticity of these cells [24, 25]. In contrast to DRIL, for which inner retinal stretching forces due to edema have been considered in its pathogenesis, ischemia resulting from damage to the blood–retina barrier in DME could affect the photoreceptor layers [20]. Therefore, DRIL is a cause of mechanical damage to bipolar cells and EZ/ELM disruption is a cause of functional damage to photoreceptors. As bipolar and Müller cells are responsible for P1 wave formation and photoreceptors are responsible for N1 and N2 formation, these structural changes may have an effect on mfERG as a functional test [26, 27].

Cystic changes within the macular area represent focal areas of accumulated extracellular fluid. These coalesced fluids are presumed to result from the damaged Müller cells, which are thought to act as water pumps to maintain the macula in its dry state. As these cells are responsible for P1 formation in mfERG, cystoid changes may have some effects on these waves [26]. Alkuraya et al. found a correlation between the severity of retinopathy and the presence of subretinal fluid with DME. They reported that serous retinal detachment was more common in severe nonproliferative diabetic retinopathy or proliferative diabetic retinopathy compared to eyes with mild to moderate nonproliferative diabetic retinopathy [27]. It has been shown that the cases with subretinal fluid had very low mfERG values [3].

Based on previous studies, visual prognosis correlates with the presence of DRIL, EZ/ELM disruption, and subretinal fluid in patients with diabetic retinopathy [12, 13]. These correlations were observed in our study. Moreover, we found a significant correlation between mfERG values, including reduced P1 and N2 amplitudes, and macular OCT changes like the presence of cysts and DRIL. There was also a correlation between a prolonged central P1 implicit time and ELM/EZ disruption. Based on these correlations, it seems that outer retinal abnormalities (ELM/EZ) affect the implicit time in mfERG while inner retinal abnormalities (DRIL and cystic changes) affect the amplitude in some waves. Zhu et al. [9] found that ELM but not EZ status had a better correlation with the latency of mfERG waves. They presumed that because of different sources of these two layers, the difference was plausible but they could not explain the reason and recommended further research to clarify the mechanism behind the difference. It is assumed that P1 is generated by Müller and bipolar cells. Therefore, mechanical disruption of bipolar or Müller cells in DRIL and cystoid macular edema may contribute to P1 amplitude attenuation. It is not clear why EZ/ELM disruption leads to P1 latency [26]. We believe that EZ/ELM makes a bridge between the inner and outer retinal layers; therefore, disruption of this bridge can delay the delivery of electrical currents to the inner retina, which may manifest as an increased implicit time. Another study found a correlation between an increased ischemic macular area and a prolonged implicit time that is present before macular edema. This implicit time prolongation may be due to ischemia induced alterations in the neuronal function [20]. Nagesh et al. [3] revealed that BCVA correlated strongly with central macular thickness, EZ, and ELM disruption in patients with diabetic retinopathy and correlated modestly with mfERG waveform amplitudes in patients with intact EZ and ELM. In contrast to our study, they found that both EZ and ELM disruption correlated modestly with N1 and P1 wave amplitudes but not with the implicit time. As more than 75% of their cases had cystoid macular edema with a significantly diminished P1 amplitude, they could not conclude the main cause of this amplitude reduction (EZ/ELM disruption or cysts) because the cysts were observed in all cases with EZ/ELM disruption in their subjects. Here, the results of this study showed a prolonged P1 implicit time after removing the effects of the cysts on mfERG parameters.

In a recent study, Das et al. found a significant correlation between disruption of the ELM/EZ, DRIL, and severity of diabetic retinopathy. They suggested that the mechanisms that created the DRIL might also be responsible for concurrent disruption of the ELM/EZ although their study was retrospective and many of their cases underwent laser therapy, which could affect the macular structure [13] The association between inner and outer retinal changes shows that it is not possible to attribute a single wave in the mfERG to a specific change in OCT; however, these mfERG parameters have a strong correlation with structural changes. As a small number of cases (only 6 cases) had simultaneous inner and outer retina abnormalities, further studies with larger sample sizes should be performed to evaluate the effect of inner and outer retina changes on one another.

Müller cells act as metabolic water pumps to keep the macula in its dry state. Disruption of Muller cells causes cystic changes in DME [26]. In our study, the presence of cysts correlated with a lower central P1 amplitude produced by bipolar and Muller cells. Yamamoto et al. [21] demonstrated that in patients with DME, the implicit time directly correlated with foveal thickness and subretinal fluid. Nagesh et al. [3] also found that subretinal fluid was accompanied by very low electrophysiology values. Our study showed that the presence of subretinal fluid in the macula resulted in a longer implicit time of N2 and N1 in the inferior and superior parts of the first ring, respectively.

One of the main limitations of this study was the small number of participants. For correlation analysis, it is difficult to detect reliable and significant results as some of these anatomical and functional factors may affect one another. Another limitation of this study was its cross-sectional design and lack of a matched control group. Hyperglycemia and high blood urea or creatinine levels have been revealed to affect the outer retina status [28, 29]. However, our patients had a good metabolic control and the patients with poor metabolic control were excluded from the study. Age is another known factor that affects mfERG parameters (e.g., amplitude reduction and implicit time prolongation). Therefore, the patients above 70 years were also excluded. Other prospective studies of the mfERG and OCT parameters changes along with DME treatment with anti-VEGFs can clarify the correlation of many of these structural and functional parameters with BCVA.

This study found that in diabetic patients, discrete changes of the mfERG parameters especially central ring P1 and N2 waves correlated with both the structural OCT abnormalities in corresponding points (like DRIL, intraretinal cysts, and EZ/ELM disruption) and BCVA. Outer retinal layer disruption (ELM and EZ) correlated with prolonged P1 implicit time at the corresponding location, and the presence of the DRIL or retinal cysts were associated with reduced P1 amplitude. Therefore, mfERG and OCT findings can complement one another to estimate the visual acuity in diabetic patients with DME. From a practical point of view, a combination of OCT and mfERG tests may provide a powerful method to identify the locus and severity of the retinal damage. However, the in-depth description of all logics behind some changes in the amplitude and implicit time of mfERG waves needs further studies with larger sample sizes.

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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 studies involving human participants 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 taken from all individual participants.

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