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Electroretinogram Changes Before and After Intra Vitreal Injection of Ranibizumab for Diabetic Macular Edema

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Short title: Electroretinogram Changes with Intra Vitreal Injection of Ranibizumab for DME.

Abstract

Background: Diabetic macular edema (DME) is a main cause of blindness globally. Vascular endothelial growth factor (VEGF) is thought to be the primary cause of DME development as a result; pharmacological therapies that suppress VEGF may have an important role in DME management. High-resolution optical coherence tomography (OCT) could be utilized to detect any change in central retinal microstructure. Multifocal Electroretinogram (mf-ERG) was used before and after intra vitreal injection of ranibizumab to detect its changes .

Objective: To evaluate the role of multifocal Electroretinogram (mf-ERG) in the follow up of DME after intravitreal Anti-VEGFs (Ranibizumab).

Methods: This prospective, case series, interventional, analytic study was held on 25 eyes of 25 patients with DME who underwent three consecutive monthly injections of IVR . Pre-operative investigations included assessment of visual acuity and best corrected visual acuity (BCVA) and slit lamb biomiocroscopy. Imaging included OCT , FFA and electroretinography (ERG) were also conducted at baseline. All cases were followed up after one week, one month, two months and three months from each injection by assessment of visual acuity (VA), best corrected visual acuity (BCVA), slit lamb biomiocroscopy (SLB), OCT and ERG. The correlation of mf-ERG values and OCT features and BCVA were investigated.

Results: There was statistically significant difference (increase) in amplitude p1 in (ring 1, ring 2 and ring 3) between baseline pre and post-treatment. There was statistically significant difference (decrease) in Implicit time PET P1 in (ring 1 ring 2 and ring 3) between baseline before and after treatment. There was statistically significant change (improvement) in macular thickness (decrease) and BCVA (increase) after treatment in comparison with pre-treatment. There was statistically significant correlation between delta amplitude and delta implicit time and (macular thickness, HBA1C and BCVA) in (Ring 1, Ring 2 and Ring 3) among studied cases ($P<0.05$).

Conclusion: The functional changes in cases with DME assessed by mf-ERG could complement OCT outcomes. Postsurgical improvement in VA was accompanied by diminished macular thickness and improved P1 amplitude and implicit time in the central ring evaluated by mf-ERG.

Keywords: Diabetic macular edema, Ranibizumab, VEGF, ERG, OCT.

INTRODUCTION

Diabetic retinopathy (DR) is a microvascular disease that occurs secondary to long-term diabetes mellitus (DM), leading to severe damage to the retina and the risk of blindness. It is the main cause of significant visual loss in working-age people in the western world[1]. Untreated DM could be accompanied by a range of eye diseases which include cataract, increased intraocular pressure (IOP), disorders of the ocular surface, recurring stye, diabetic papillopathy, and DR. DR is the most common and severe eye condition associated with DM. DR deteriorates due to inadequate management of blood sugar levels, uncontrolled

hypertension, abnormal lipid levels, kidney disease, being male, and obesity[2].

Around twenty-seven percent of individuals exhibit signs of macular edema within 9 years after being diagnosed with DM[3]. DME is a prominent factor in global visual impairment. Around 100 million individuals worldwide exhibit symptoms of macular edema because of diabetes. Research indicates that around one in three individuals with diabetes exhibit signs of macular edema[4]. DME is more common in patients with DM type 1 than in those with DM type II[4].

The increased prevalence of diabetes in industrialized countries may contribute to a rise in the absolute prevalence of DME. The incidence of DME is expected to decrease as patients and medical practitioners come to understand the importance of superior metabolic control as a therapeutic one[5].

Various pharmacologic treatments, particularly anti-VEGF drugs and corticosteroids, are now accessible for treating DME. VEGF is a powerful agent that increases blood vessel permeability and promotes the growth of new blood vessels. Elevated levels of VEGF in the vitreous have been seen[6]**.** IVI of anti-VEGF are the most efficient therapy for DME affecting the central area of the eye. These agents involve pegaptanib, ranibizumab, aflibercept, and bevacizumab[7].

The intravitreal injection (IVI) of anti-VEGF agents is considered the classic therapeutic modality for retinal disorders as age-related macular degeneration (AMD), DME, & ME caused by RVO[8]. Ranibizumab which is authorized by the FDA for treating diabetic macular edema , is a 48-kD Fab fragment which binds to the biologically active VEGF-A receptors, such as VEGF-110 which blocks the binding of VEGF-A to VEGFR1 and VEGFR2 receptors on endothelial cells[9]**.** Electroretinogram (ERG) is the retina's electrical response to light stimulation[10].

Full-field flash ERG and mfERG are two important diagnostic techniques that detect functional alterations in the retina. mfERG was created by Sutter and Tran in 1992 as a method to capture the electrical responses from several areas of the retina[11]. It allows a quick evaluation of retinal function from many regions simultaneously by employing a stimulus that alternates between high and low contrast[12].

Analyzing the components of the mfERG waveform might offer valuable diagnostic insights for differentiating between different anterior visual pathway illnesses, particularly when the cause of vision impairment is still unclear after a routine clinical evaluation[13]. So, we aimed to assess ERG changes before and after IVI of Ranibizumab (IVR) for DME and its correlation with macular thickness.

PATIENT AND METHOD

This prospective, case series, interventional, analytic study was held in Mansoura university ophthalmic center on 25 eyes of 25 patients attended to Mansoura University Ophthalmic Center with DME undergoing intra vitreal injection of ranibizumab in the period from May 2023 to May 2024.

This study included patients aged over 18 years old from both genders with BCVA not worse than 1.5 log-MAR with significant DME in need of intravitreal injection of ranibizumab and central retinal thickness (CRT) was evidenced > 250 μm by OCT. But we excluded cases with vascular occlusive disorders, with bleeding coagulopathies, with inflammatory retinal diseases, with previous retinal surgery, with media opacity which doesn't permit OCT acquisition with good signal strength or with macular ischaemia or disturbance of the foveal avascular zone (FAZ) in fundus fluorescein angiography (FFA). Patients with high HbA1c indicating poor control of diabetes are excluded till they reach at least level of fair control .

Methods

Every patient was subjected to thorough history taking including demographic data (age, sex, history of preceding intraocular surgery, neurological, metabolic or systemic disorders).

Ophthalmic examination comprised VA assessment involving VA (visual acuity) and BCVA by using Landlots' broken ring chart then converted to logMAR**.** Anterior segment examination was done by SLB (Haag Streit BP 900, Haag-Streit, Koeniz, Switzerland) to evaluate corneal clarity, anterior chamber depth, state of iris, pupillary reaction, shape, regularity and morphology of the lens. Refraction was assessed by automated refractometer.

Goldman applanation tonometry was used for IOP measurement. Fundus examination was done by SLB using

non-contact Volk lens +78D or +90D to assess the degree of DME and any vascular disorder or disc disorders. FFA was done for all patients to exclude macular ischemia. OCT was utilized for central macular thickness (CMT).

Electroretinography (ERG)

It's used to measure the electric retinal activity in response to a light stimulus. mf-ERG was taped using RETI-scan (Ronald Consult, RETI port/scan21, SN 04-99-8022).

Steps of Electroretinography[11]

The pupils should be maximally dilated. Mydriatic eye drops three times within half an hour were utilized for maximum pupil dilatation prior to ERG. The recordings were conducted under room light situations and prior to OCT to prevent retinal cells saturation. The mfERG was conventionally reported in photopic situations. This excluded rod participations in the signal and ensures a cone-driven response primarily. The patient's chin was positioned in the chin rest. Using binocular recording was advised since it enhanced fixation stability and reduces examination time. Recording Electrodes were positioned on the bulbar conjunctiva nearby to the inferior limbus following one drop of 0.5% proparacaine HCl.

Reference Electrodes were positioned on the skin onto the temporal area one cm ahead of the outer canthus of each ipsilateral eye. Ground Electrodes were typically placed over the supraorbital edges at the midline of the forehead and connected to the "ground input" of the recording system. Before placement of the ground electrode and the reference electrode, the relevant skin area was sterilized by ethanol to clean the superficial skin layer and the fatty layer identified to have low electric conductivity. Recording of the signal was achieved by combining the electrodes via the connection box.

Intravitreal injection (IVI) of Ranibizumab (IVR)

Therapeutic protocol included IVI of Ranibizumab (0.05 ml of a 0.23 ml/2.3 mg per vial) via the pars plana once every month for three months. Prophylactic topical antibiotic (Vigamox ED Q.I.D.) was given on the day before the operation. One hour prior to surgery, pupillary dilatation was done by using mydriatic eye drops every ten minutes for half an hour preoperatively. Then Ranibizumab (Lucentis) (Novartis/ Switzerland) was supplied as a preservative-free, colorless to pale yellow, and sterile solution. The dose was 0.05 ml of a 0.23 ml/2.3 mg per vial of Ranibizumab.

Follow up and Outcomes

The follow-up was 24 hours and one week following IVI and after that monthly for three months. Outcomes comprised BCVA, mf ERG P1 amplitude and implicit time (functional response) and central macular thickness. Patient responses were classified based on change in BCVA into good response in cases gaining more than 2 lines on snellen chart, moderate response in cases gaining less than 2 lines and poor response in cases demonstrating stable vision on chart.

In the current study prognostic factors for short-term visual and anatomic improvement of intravitreal ranibizumab (IVR) and mfERG changes for DME including BCVA, central macular thickness, age, side of affected eye and HbA1C were evaluated aiming to detect the best parameters that can predict patient response.

Statistical Analysis

Data analysis was conducted by SPSS software (PASW, version 25 Chicago: Inc.). Qualitative data were described using numbers and percentages. Quantitative data were defined using mean±SD for normally distributed data following testing normality using Kolmogrov-Smirnov test. The significance of the obtained results was judged at the ($\leq$0.05) level. Repeated Measures ANOVA test was utilized to compare more than 2 paired readings with Post Hoc Tukey test to detect pair-wise comparison. Paired t-test was used to compare pre and post treatment values.

RESULTS

This study included 25 eyes of 25 patients with DME collected from outpatient clinics in Mansoura ophthalmic center after they had met inclusion/exclusion criteria.

Table 1: Demographic characteristics, Affected side, and disease characters of the studied cases

	N=25	**%**
Age / years	58.44±5.77	
Sex		
Male	11	44.0
Female	14	56.0
Side		
Right	11	44.0
Left	14	56.0
DM type		
IDDM	17	68.0
NIDDM	8	32.0
Lens		
phakic	21	84.0
pseudophakic	4	16.0
Duration (years)	12.08±2.58	

IDDM: Insulin dependent diabetes mellitus NIDDM: Non insulin dependent diabetes mellitus

Table (1) demonstrates that mean age of studied cases was 58.44±5.77 years, 56% of the cases were females and 44% were males. mean duration of disease in studied cases was 12.08±2.58 years, 56% of the cases were LT-sided affected and 44% were RT-sided affected. DM type was IDDM in 68% of the cases and NIDDM in 32%. Lens was phakic in 84% and pseudophakic in 16% of the cases.

Table 2: Change of macular thickness, BCVA, HbA_1C after treatment as compared to before treatment

	Before	**After**	**Mean difference (95%CI)**	**P value**
Macular thickness	347.52±41.87	273.0±31.69	74.52 (53.92,95.12)	0.001*
BCVA	0.984±0.233	0.774±0.215	0.209 (0.093-0.325)	0.001*
HbA1c	8.02±0.37	6.96±0.782	1.072 (0.742-1.41)	0.001*

BCVA : best corrected visual acuity

Data expressed as mean ±SD

CI: Confidence interval, used test: paired t-test, *statistically significant

Table **(2)** shows statistically significant change in macular thickness and BCVA post-treatment as compared to pre-treatment (P=0.001), where there is decrease in macular thickness and increase in BCVA after treatment. There was statistically significant change in HbA1c post-treatment as compared to pre-treatment (P=0.013), where there was decrease in HbA1c indicating good control of most of diabetic patients during study.

Table 3: Comparison of amplitude p1 change during different follow ups

Amplitude P1 (NV/DEG2)	**Before**	**After 1st**	**After 2nd**	**After 3rd**	**Overall p value**
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	
Ring1	25.88±18.22	34.49±17.86	36.95±17.21	42.75±21.01	F=4.35 P=0.007*
#Comparison with baseline		-8.62 (-15.23, -1.99) ***P=0.013****	-11.07 (-20.38, -1.76) ***P=0.02****	-16.86 (-29.41, -4.32) ***P=0.01****	
#Comparison with After 1st			-2.45 (-10.53,5.62) ***P=0.536***	-8.25 (-20.92, 4.42) ***P=0.192***	
#Comparison with After 2nd				-5.79 (-13.73, 2.14) ***P=0.145***	
Ring2	18.81±7.78	22.11±8.73	24.21±9.62	30.61±11.63	F=9.59 P=0.001*
#Comparison with baseline		-3.30 (-7.32, 0.725) ***P=0.104***	-5.40 (-10.11, -0.70) ***P=0.026****	-11.80 (-18.04,- 5.56) ***P=0.001****	
#Comparison with After 1st			-2.10 (-5.78, 1.58) ***P=0.251***	-8.49 (-13.41, -3.58) ***P=0.002****	
#Comparison with After 2nd				-6.39 (-10.49, -2.3) ***P=0.004****	
Ring3	13.94±5.31	15.54±6.28	17.38±6.95	21.22±8.78	F=6.83 P=0.001*
#Comparison with baseline		-1.59 (-4.47, 1.27) ***P=0.261***	-3.43 (-6.52, -0.36) ***P=0.03****	-7.28 (-12.15, -2.41) ***P=0.005****	
#Comparison with After 1st			-1.84 (-4.59, 0.915) ***P=0.181***	-5.68 (-9.31,-2.05) ***P=0.004****	
#Comparison with After 2nd				-3.84(-7.19, -0.487) ***P=0.027****	

Comparison include mean difference (95%CI), P value

Data expressed as mean ±SD, CI: Confidence interval, used test: paired t test, *statistically significant, F: Repeated Measures ANOVA test

Table **(3)** displays a significant difference in amplitude p1 in (ring 1, ring 2 and ring 3) between baseline before treatment and after (1st month, 2nd month and 3rd month of treatment (P=0.007, 0.001 and 0.001, respectively). There is statistically significant difference in amplitude p1 in (ring 1, ring 2 and ring 3) (between baseline and after (1st month)), (between baseline and after (2st month)) (between baseline and after 3rd month of treatment), (between 1st month and (2st and 3rd months) and (between 2st and 3rd months), where there is an increase in amplitude p1 in (ring 1, ring 2 and ring 3) in follow-up months after treatment.

Table 4: Comparison of Implicit time PET P1 change during different follow ups

Implicit time PET P1(ms)	**Before Mean ±SD**	**After 1st Mean ±SD**	**After 2nd Mean ±SD**	**After 3rd Mean ±SD**	**Overall p value**
Ring1	57.76±6.37	55.45±4.48	54.32±7.95	52.28±11.21	F=4.48 P=0.006*
Comparison with baseline		2.31 (0.151-4.47) ***P=0.037****	3.44 (-0.109,6.99) ***P=0.057***	5.48 (0.638-10.32) ***P=0.028****	
Comparison with After 1st			1.12 (-0.826, 3.08) ***P=0.245***	3.17 (-0.284. 6.62) ***P=0.07***	
Comparison with After 2nd				2.04 (0.288-3.79) ***P=0.024****	
Ring2	55.42±4.32	53.09±2.48	52.44±3.60	51.32±5.35	F=6.71 P=0.001*
Comparison with baseline		***2.32(0.675-3.98) P=0.008****	***2.98(0.732-5.22) P=0.01****	***4.10(1.04-7.16) P=0.01****	
Comparison with After 1st			***0.652(-0.464, 1.76) P=0.240***	***1.78(-0.07, 3.63) P=0.06***	
Comparison with After 2nd				***1.124(0.110-2.14) P=0.03****	
Ring3	52.90±2.99	51.24±2.75	49.62±2.60	47.69±2.97	F=6.83 P=0.001*
Comparison with baseline		***1.66 (1.24-2.41) P=0.001****	***3.28 (2.52, 4.05) P=0.001****	***5.21 (4.08, 6.34) P=0.001****	
Comparison with After 1st			***1.62(1.22, 2.0) P=0.001****	***3.54(2.77, 4.31) P=0.001****	
Comparison with After 2nd				***1.92(1.52- 2.33) P<0.001****	

Comparison include mean difference (95%CI), P value

Data expressed as mean ±SD, CI: Confidence interval, used test: paired t test, *statistically significant, F: Repeated Measures ANOVA test

Table (4) shows statistically significant difference in Implicit time PET P1 in (ring 1, ring 2 and ring 3) between baseline before treatment and after (1st month, 2nd month and 3rd month of treatment (P=0.006 , 0.001 and 0.001, respectively) where there was a decrease in Implicit time PET P1 in (ring 1 , ring 2 and ring 3) in follow-up months after treatment.

Table 5: Amplitude P1 frequency and Implicit time of increase and decrease before and after treatment

	Change			
	Significant increase		**Non Significant increase**	
	N	%	N	%
Amplitude P1 (NV/DEG2)				
Ring1	22	88.0	3	12.0
Ring2	22	88.0	3	12.0
Ring3	22	88.0	3	12.0
	Change			
	Significant decrease		**Non significant decrease**	
	N	**%**	**N**	**%**
Implicit time PET P1(ms)				
Ring1	22	88.0	3	12.0
Ring2	22	88.0	3	12.0
Ring3	22	88.0	3	12.0

Table **(5)** shows that Amplitude P1 frequency of significant increase after treatment is 88% in (ring 1, ring 2 and ring 3) and Amplitude P1 frequency of non significant increase after treatment is 12% in (ring 1, ring 2 and ring 3). The Implicit time PET P1 frequency of non significant decrease after treatment was 12% in (ring 1, ring 2 and ring 3) and Implicit time PET P1 frequency of significant decrease after treatment is 88% in (ring 1, ring 2 and ring 3).

Table 6: Correlation between delta amplitude, delta implicit time and macular thickness, HBA1C and BCVA among studied cases

		Delta amplitude			Delta implicit time		
		Ring 1	Ring2	Ring 3			
Delta macular thickness	r	-.557	-.578	-.701	.736	.553	.669
	p value	**.004***	**.002***	**.001***	**.001***	**.004***	**.001***
	N	25	25	25	25	25	25
Delta HBA1c	r	-.564	-.553	-.615	.522	.617	.493
	p value	**.003***	**.004***	**.001***	**.007***	**.001***	**.012***
	N	25	25	25	25	25	25
Delta BCVA	r	-.748	-.609	-.580	.751	.653	.771
	p value	**.001***	**.001***	**.002***	**.001***	**.001***	**.001***
	N	25	25	25	25	25	25

r: Spearman correlation coefficient, *statistically significant

Table **(6)** shows statistically significant correlation between delta amplitude and (macular thickness, HBA1C and BCVA) in (Ring 1, Ring2 and Ring 3) among studied cases (P<0.05), also there was statistically significant correlation between Delta implicit time and (macular thickness, HBA1C and BCVA) in (Ring 1, Ring2 and Ring 3) among studied cases (P<0.05).

Case study

Male patient 56 years old diabetic, phakic with diffuse macular oedema with CMT 410 µm. Gradual reduction of CMT one week after the 3rd IVR to 219 µm. P1 amplitude of central rings increase gradually as follow: ring (1) 18.7 [nv/deg2], ring (2) 14.3 [nv/deg2], ring (3) 9.8 [nv/deg2] before injection to reach ring (1) 46.7 [nv/deg2], ring (2) 40.5 [nv/deg2], ring (3) 28.1 [nv/deg2] one week after the third injection. PeT P1of central rings decrease gradually as follows: ring (1) 59.8 [ms], ring (2) 54.9[ms], ring (3) 53.9 [ms] before injection to reach ring (1) 57.8[ms], ring (2)53.9[ms], ring (3) 50.0 [ms] one week after the third injection. HbA1c before the study was 10.9 and with good control during study, it reached 6.8 one week after the 3rd injection. BCVA improved from basal value logMAR 1.3 to 0.8 seven days after the 3rd injection (Figs 1-6).

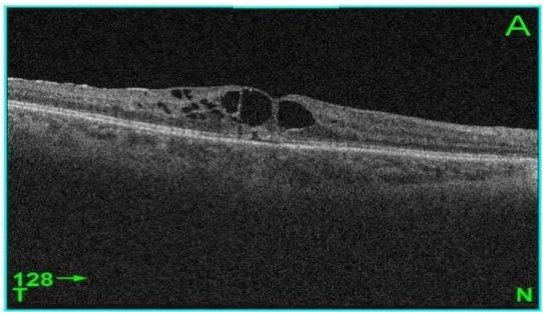


Figure (1): OCT pre-injection

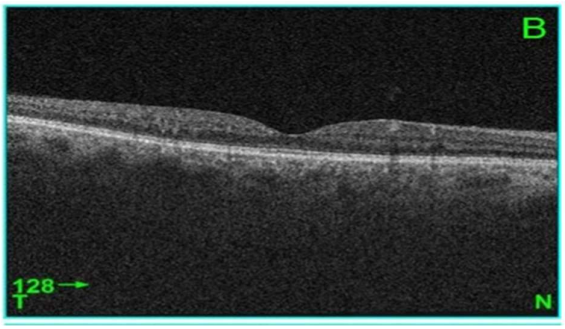
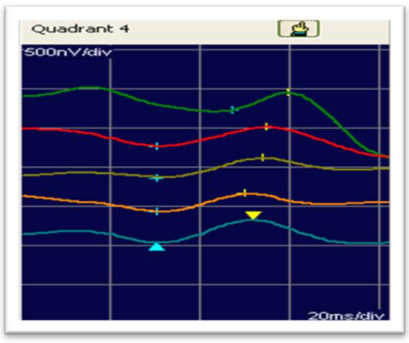


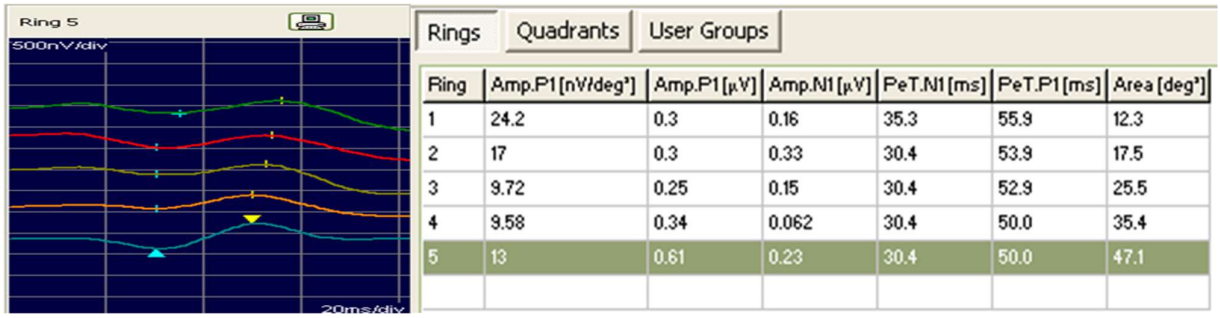
Figure (2): OCT after the 3rd injection



Rings | Quadrants | User Groups

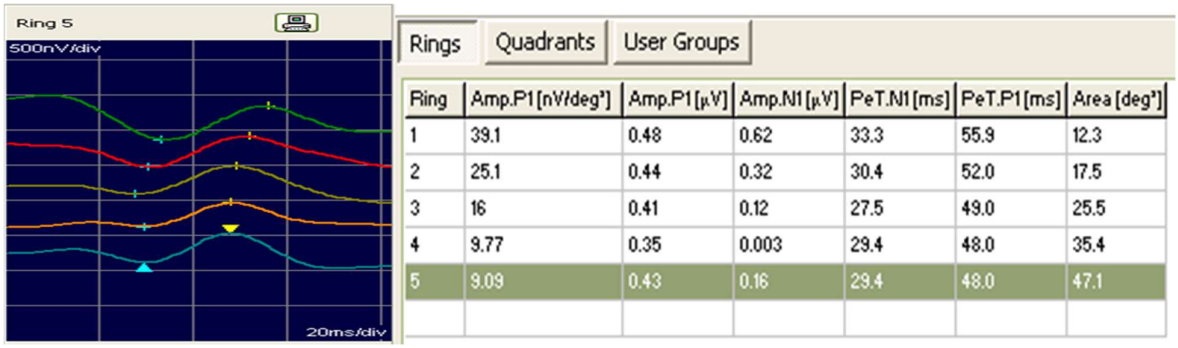
Ring	Amp.P1 [nV/deg²]	Amp.P1 [µV]	Amp.N1 [µV]	PeT.N1 [ms]	PeT.P1 [ms]	Area [deg²]
1	18.7	0.23	0.21	47.1	59.8	12.3
2	14.3	0.25	0.23	30.4	54.9	17.5
3	9.8	0.25	0.032	30.4	53.9	25.5
4	6.73	0.24	0.18	30.4	50.0	35.4
5	6.43	0.3	0.14	30.4	52.0	47.1

Figure (3) : mfERG pre-injection



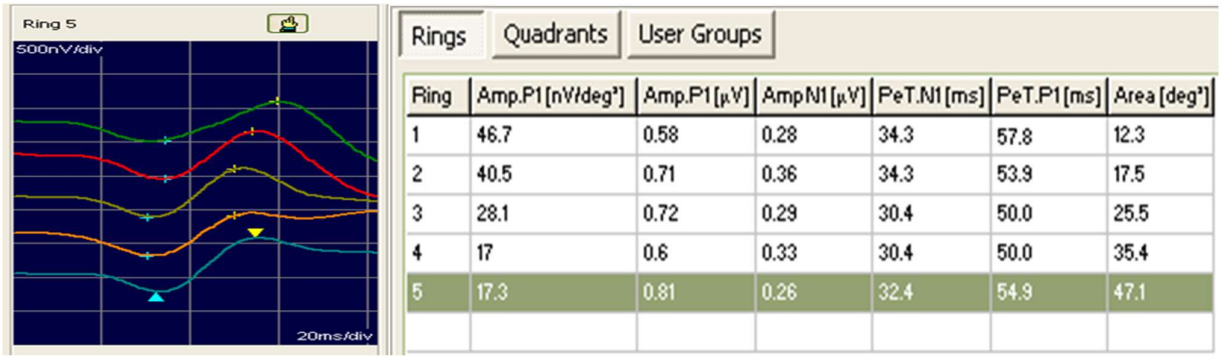
Ring	Amp.P1 [nV/deg²]	Amp.P1 [µV]	Amp.N1 [µV]	PeT.N1 [ms]	PeT.P1 [ms]	Area [deg²]
1	24.2	0.3	0.16	35.3	55.9	12.3
2	17	0.3	0.33	30.4	53.9	17.5
3	9.72	0.25	0.15	30.4	52.9	25.5
4	9.58	0.34	0.062	30.4	50.0	35.4
5	13	0.61	0.23	30.4	50.0	47.1

Figure (4): **mfERG** after the 1st injection



Ring	Amp.P1 [nV/deg²]	Amp.P1 [µV]	Amp.N1 [µV]	PeT.N1 [ms]	PeT.P1 [ms]	Area [deg²]
1	39.1	0.48	0.62	33.3	55.9	12.3
2	25.1	0.44	0.32	30.4	52.0	17.5
3	16	0.41	0.12	27.5	49.0	25.5
4	9.77	0.35	0.003	29.4	48.0	35.4
5	9.09	0.43	0.16	29.4	48.0	47.1

Figure (5) : **mfERG** after the 2nd injection



Ring	Amp.P1 [nV/deg²]	Amp.P1 [µV]	Amp.N1 [µV]	PeT.N1 [ms]	PeT.P1 [ms]	Area [deg²]
1	46.7	0.58	0.28	34.3	57.8	12.3
2	40.5	0.71	0.36	34.3	53.9	17.5
3	28.1	0.72	0.29	30.4	50.0	25.5
4	17	0.6	0.33	30.4	50.0	35.4
5	17.3	0.81	0.26	32.4	54.9	47.1

Figure (6) : **mfERG** after the 3rd injection

Fig. : 1: OCT pre-injection, **2**: OCT after the 3rd injection, **3: mfERG** pre-injection, **4**: **mfERG** after the 1st injection, **5 mfERG** after the 2nd injection, **6**: **mfERG** after the 2nd injection

DISCUSSION

Diabetic retinopathy (DR) is a main cause of visual loss globally, and DME is a main adverse event of DR that causes blindness. VEGF is signaling protein that triggers DME development as a result, pharmacological treatments that suppress VEGF could have an important role in DME management [14].

Ranibizumab is a human anti-VEGF monoclonal antibody, utilized in association with all VEGF-A active isomers. A small phase I clinical trial of the IVR for treatment of DME demonstrated that the patients' state improved, and there were no ocular or systemic adverse reactions recorded at the termination of the treatment[15].

The anatomical improvement may be assessed via OCT. On the other hand, in spite of the anatomic success and reduce of macular oedema, some cases have a poor functional recovery[16].

Also, mf-ERG helps us to evaluate the retinal electrophysiological activity of the retina and presents a topographic map. It could report focal ERG responses concomitantly from various areas in the central 40° to 50° of the retina. It is used to get an electrophysiological response from the cones after the light adaptation [17].

Therefore, the current study aimed to study the mf-ERG role in the follow-up of DME following intravitreal injection of anti-VEGFs (Ranibizumab).

The current study enrolled 25 eyes of cases complaining from DME without macular ischaemia. In this study, we observed that the response density of P1 significantly increased over follow-up time in the central three rings after the treatment. At the same time, we also found that as macular thickness decreased, the P1 response density increased. OCT is a very useful and frequently used imaging method in showing the anatomical structure of edema, but it is insufficient in evaluating the damage caused by edema to cells. mf-ERG is an imaging method that through concurrent stimulation of various areas of the retina, the retinal function can be mapped in the macula.

The current study showed statistically significant change in macular thickness and BCVA post-treatment in comparison with pre-treatment (P=0.001) where there was decrease in macular thickness and increase BCVA after treatment.

Similarly, **Nepomuceno et al.** conducted their study on a total of 63 eyes with center comprising DME and were haphazardly allocated to receive 1.5 mg bevacizumab or 0.5 mg ranibizumab at baseline and repeated every month if the central sub-foveal thickness was more than 275 µm. BCVA was significantly improved in both groups at all visits [19]. Also, **Tawakol et al.,** noticed that IVI of anti-VEGF significantly improved BCVA from (0.88±0.12) preoperatively to (0.53±0.18) at the end of the 3rd month, with P< 0.001. In addition, significant improvement was recorded in foveal thickness from preoperative to three months postoperative (408.73±79.40µm versus 224.33±32.49 µm respectively) ($p < 0.001$)[18].

This study showed that VA increased in all cases that it's implicit times significantly decreased after the three injections.

Holm et al., recorded that VA increased following IVR, and demonstrated that implicit times significantly diminished following the IVR[20].

Also, the current study showed statistically significant relation between the strict control of diabetes measured by HbA1c and the improvement of BCVA during course of IVR. It has been demonstrated that blood glucose levels could interfere with mf-ERG outcomes [21]. The current study found statistically significant difference in amplitude p1 in (ring 1, ring 2 and ring 3) between baseline before treatment and after (1st month, 2nd month and 3rd month of treatment (P=0.007, 0.001 and 0.001, respectively). There was statistically significant difference in amplitude p1 in (ring 1, ring 2 and ring 3) (between baseline and after (1st month)), (between baseline and after (2nd month)) (between baseline and after 3rd month of treatment), (between 1st month and (2nd and 3rd months) and (between 2nd and 3rd months), where there was an increase in amplitude p1 in (ring 1, ring 2 and ring 3) in follow-up months after treatment. Additionally, the current study found statistically significant difference in Implicit time PET P1 in (ring 1 , ring 2 and ring 3) between baseline before treatment and after (1st month, 2nd month and 3rd month of treatment (P=0.006 , 0.001 and 0.001, respectively). There was statistically significant difference in amplitude p1 in (ring 1, ring 2 and ring 3) (between baseline and after (1st month)), (between baseline and after (2nd month)) (between baseline and after 3rd month of treatment), (between 1st month and (2nd and 3rd months) and (between 2nd and 3rd months), where there was progressive decrease in Implicit time PET P1 in (ring 1, ring 2 and ring 3) in follow-up months after treatment.

So, Amplitude P1 frequency of significant increase after treatment in the current study was 88% in (ring 1, ring 2 and ring 3) and Amplitude P1 frequency of non-significant increase after treatment was 12% in (ring 1, ring 2 and ring 3). While, Implicit time PET P1 frequency of non-significant decrease after treatment was 12% in (ring 1, ring 2 and ring 3) and Implicit time PET P1 frequency of significant decrease after treatment was 88% in (ring 1, ring 2 and ring 3).

Fu et al., assessed MF-ERG alterations in 27 eyes of cases with DME following the administration of IVR in three successive injections (evener month) and as required thereafter. At every assessment, there was a significant increase in the mean amplitude of P1 in the center ring when compared to the baseline. On the other hand, the mean P1 implicit time in the central ring was reduced without reaching statistical significance. Significant association were recorded between BCVA and both CMT and P1 amplitude in the central retina. They recorded that, together with the improved BCVA and the decreased CMT, IVR improved macular retinal functions, as revealed by mf-ERG, in the context of diabetic eyes[22].

Sivaprasad et al., [23] set up a comparison between laser photocoagulation and anti-VEGF and demonstrated that anti-VEGF could achieve better corrected VA; with the minimal possibility of a center comprising macular oedema and vitreous haemorrhage recorded in the anti-VEGF group compared to the laser photocoagulation group

Specifically, our study found statistically significant association between delta amplitude and (macular thickness, HBA1C and BCVA) in (Ring 1, Ring2 and Ring 3) among studied cases ($P<0.05$) and statistically significant association between Delta implicit time and (macular thickness, HBA1C and BCVA) in (Ring 1, Ring2 and Ring 3) among studied cases ($P<0.05$).

Yamamoto et al., revealed that mf-ERG results from the macula could be considered a promising indicator of macular functions among cases with DME and had a strong correlation with morphological alterations in the macula[24].

In agreement with our findings, **Tawakol et al.,** displayed that there was a significant reduction in P1 amplitude among cases with DME. Through the observations made throughout a short period of three months following IVI of anti-VEGF, P1 amplitude P1 was most directly linked to the BCVA with the reduction in the CMT. This revealed that IVI of anti-VEGF has the ability to reduce DME and aid in the recovery of inner retinal cell function[18].

Preceding researches displayed IVI of anti-VEGF effects on mf-ERG in DME and the increments of P1 amplitudes in the central ring with IVR treatment[25, 26]. Significant associations between BCVA and mf-ERG amplitude were recorded in preceding researches of maculopathies[27]. **Kaderli et al.,** found a positive relationship between VA and the P1 amplitude was accomplished at six months of the treatment[16].

The discrepancies in the outcomes of the aforementioned studies could be explained by the retrospective nature of some studies, dissimilar populations, different follow-up periods, the selection of cases, and the small sample size.

CONCLUSIONS

The functional changes in cases with DME assessed by mf-ERG could complement OCT outcomes. Postsurgical improvement in VA was accompanied by diminished macular thickness and improved P1 amplitude and implicit time in the central ring evaluated by mf-ERG.

RECOMMENDATIONS

mf-ERG could be used in the follow up of DME who undergoing IVI . Long-term investigations and larger sample sizes are needed for more reliable documentation.

Declarations

Conflict of Interest

None.

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Declaration of Interest

No financial affiliations with any organizations.

Consent for publication

Not applicable

Availability of Data

This study contains all of the data generated during this review..

Standards of Reporting

CONSORT guidelines were followed.

Author's contributions

All authors read and approved the final manuscript after proper interpretation and discussion.

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