Sensitivity and specificity of multifocal and full-field electroretinography in diabetic retinopathy

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Abstract-Non-invasive recordings of the retinal activity have an important role in the diagnosis of retinal pathologies and in defining the state of retina. The current study described the responses of F-ERG and MF-ERG in patients with diabetic retinopathy (DR) compared to normal subjects, to establish whether there were differences in MF-ERG first order response among normal, NPDR, and PDR eyes, to correlate their results in detecting dysfunction in patients with diabetic retinopathy and to determine their sensitivity and specificity. Twenty patients with DR and 20 eyes of 10 normal subjects were examined using MF-ERG and F- ERG. The latencies and amplitudes were measured, recorded and compared among the three groups. The mean and standard deviation (SD) were evaluated using statistical package for social science (SPSS.15). Receiver operator characteristic (ROC) curves were constructed to determine the sensitivity and specificity of abnormal values in patients compared to the normal controls.The results of this study showed that Diabetic retinopathy (DR) markedly affected on all parameters of MF-ERG. The response densities of MF-ERG were decreased and latencies of p-wave were prolonged. The MF-ERG responses obtained from eyes with DR were significantly different (P > 0.05) from those of normal eyes.in F-ERG latencies of a-waves photopic response and 30HZflicker were not different among the groups. In DR significant correlation found between ring 5 and standard combined response amplitudes and latencies and also with cone response. In non-proliferative diabetic retinopathy, the areas under ROC curves (AUCs) were larger for the MF-ERG (0.648 to 0.857) than those for the F-ERG (0.457 to0.573) which means that MF-ERG responses yielded greater sensitivity and specificity than F-ERG. In proliferative diabetic retinopathy, there was no difference in the AUCs and sensitivities between MF-ERG and F-ERG.

Keywords: Diabetic retinopathy, Full field electroretinography, multifocal electroretinography, sensitivity of MF-ERG and F-ERG in DR.

Abbreviations: DR: Diabetic Retinopathy; NPDR: Non-Proliferative Diabetic Retinopathy; PDR: proliferative diabetic retinopathy; F-ERG: Full field Electroretinogram; MF-ERG: multifocal Electroretinogram; ISCEV – International Society for Clinical Electrophysiology of Vision

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1 INTRODUCTION

Diabetic retinopathy (DR) is one of the most common complications of diabetes and is the leading cause of vision loss in working-age adults. Retinopathy remains a serious health problem, accounting for 8% of all cases of blindness in the United States [1]. The 2012 global estimate that there are approximately 93 million people living with DR and among them 28 million are with vision threatening DR [2]. Additionally, symptoms often do not appear in the early stages of DR, making prevention and early treatment more challenging, especially in the population with undiagnosed diabetes. DR has classically been defined as pathology of the microvasculature, primarily of the inner retina [3]. DR can be classified into two sub-classifications non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).

The earliest form is non- proliferative diabetic retinopathy (NPDR), also referred to as background or simple diabetic retinopathy. In this form, there is abnormal dilation of blood vessels, leakage and bleeding of the blood vessels, and fluid accumulation within the retina. A more advanced form, (PDR), is more sight-threatening. It is characterized by neovascularization, the formation of abnormal new blood vessels that are fragile and leaky. PDR is the primary cause of severe vision loss in diabetes [4]. Diabetic patients are assessed using ophthalmoscopy and fundus photography [5, 6]. The main focus is to detect visible sign of vascular retinopathy in order to monitor progress of DR and to avoid its sight-threatening complications [7, 8]; however the basis of functional changes in the retina, especially in the early stages, has not been determined.

The study of the electrical nature of biological cells and tissues is the basis of several ophthalmological techniques that can provide information about the retinal function. Three distinct electrical potentials have been identified in retina are early receptor potential (ERP), electroretinography (ERG), and electrooculogram (EOG). ERP is generated by the photoreceptors in the outer retina.EOG is function of pigment epithelium but also depends on outer and inner layers of the retina. ERG generation extends from pigment epithelium to the inner nuclear layer. [9]

Full-field electroretinography (F-ERG) is the diffuse response of both neural and non neural cells of the retina to a light stimulus. The response recorded after such a stimulus represents the sum of the positive and negative components that originate from different stages of retinal processing overlap in time. The recorded electrical activity is the result of light-induced changes in the transretinal movements of ions, principally sodium and potassium, in the extracellular space. The electrical responses originated in the retina are recorded by active electrodes that contact the cornea or nearby bulbar conjunctiva.

The ERG generally consists of a negative deflection called the a-wave and a positive deflection called the b-wave. A-wave is mainly a response of photoreceptors, while the b-wave is mainly associated with on-bipolar cell function (The a-wave in standardized ERG is generated by the rod phototransduction. The b-wave is arises from the ON bipolar cell depolarization after the signal originated in the outer cell membranes of the photoreceptor). A-wave is generally not recordable in rod ERG responses recorded under low-intensity flashes. There is a large magnification of electrical activity as signals are transmitted from Photoreceptors to inner retinal neurons, for this reason, b-wave may be recorded in lower intensity flashes which does not produce a-wave response. However, as the intensity of the light stimulus increases, the a-wave begins to be recorded. But, in such intensities, the cone photoreceptors begin to respond to the light flash. This violates the recording of absolute rod photoreceptor function. For this reason, b-wave response in rod ERG shows the on-bipolar cell function originated from the rod photoreceptors. Standard flash stimulates both rod and cone photoreceptor and generates both a- and b-wave deflections. Oscillatory potentials are a series of wavelets on the ascending limb of the ERG b-wave after stimulation by an intense light flash. These are high-frequency, low-amplitude components of the ERG with a frequency of about 100 to 160 Hz. These responses originate in the circuitry between the amacrine cells and other retinal neurons. By comparison, the a- and b-waves are dominated by frequency components of about 25 Hz [10]. Single-flash cone ERG and 30-Hz flicker ERG have a-and b-wave components representing the cone photoreceptors and cone on-bipolar cell function respectively.

The (F-ERG) has been used to study retinal functional changes in diabetic patients [11-12]. The defects of DR are not distributed uniformly across the retina, and show a range of stages of development [13]. The (F-ERG), which is a summated retinal response measurement, is not likely to reflect local or eccentric functional changes in diabetes. The multifocal electroretinogram (MF-ERG) is a relative new diagnostic method that was first introduced by Sutter and Tran (1992). This method provides objective topographical measurements of retinal responses across the visual field [14].

2 SUBJECTS AND METHODS:

The study was carried out in Mansoura Ophthalmic Center after obtaining approval of Mansoura Ophthalmic Ethic committee. Informed consents were also obtained from all participating subjects after they were give explanation of the study. The study included two groups: First group included 10 healthy individuals (3 males, 7females) aged between 43 and 58 years with no abnormalities of the visual system. Second group included 20 patients (9 males, 11 females) with Diabetic retinopathy aged between 45 and 60 years. Diabetic retinopathy subjects were further subdivided into 2 subgroups: proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy (NPDR). The level of retinopathy was determined for each patient on the basis of results of fluorescein angiography. Both eyes of each subject were tested using full - field and multifocal electroretinogram.

The exclusion criteria included poor central or unsteady fixation of eyes, poor cooperation, and any other ocular diseases including fundal problems.

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Electroretinogram

Roland consults Brandenbrug, Germany instrument was used to record standard full field ERG and multifocal ERG. The pupils were dilated with tropicamide eye drop 1% before the electrophysiological examination. Responses were recorded by using Dawson Trick-Litzkow (DTL) electrodes. Positive electrodes were placed in the lower fornix of each eye and fixed temporally. Gold- cup reference and surface electrodes were applied to the subjects' temple and forehead, respectively. . (fig1.a)

Fullfield ERG

Full field ERG was performed on both eyes simultaneously. Five steps were done. After 30 minutes of dark adaptation the subject put the head on Ganzfeld stimulator(fig1.b), 3steps were recorded (rod-response, combined response and oscillatory potential).After scotopic measurements, photopic recordings were preceded by a light adaptation of 10 minutes to a background light of 30 cd/m^2.In photopic recording two steps were recorded(cone response and 30 HZ flicker). The responses were digitally band-pass filtered from 0.5 to 1000Hz.

Multifocal MfERGs

The stimulus, consisting of 61scaled hexagonal elements covering a central visual field of 60° × 55°, were presented on a 19-inch. Monitor at a frame rate of 75 Hz at a distance of 32 cm from the subject's eyes. The size of the hexagons was scaled eccentricity to elicit approximately equal amplitude responses at all location. Each hexagon was temporally modulated between black and white according to pseudo-random binary m-sequence with luminance of 100cd/m2 in white hexagons and 2cd/m2 in black hexagons (fig1.c). DTL fiber electrodes were applied to both eyes, waveforms were recorded, amplified (200,000×) and band pass-filtered (10–100 Hz).subjects were optically corrected for the viewing distance and were asked to maintain fixation on the red fixation target at the center of stimulus matrix and refain from blinking. Recording artifacts due to blinking or small eye movement were detected and discarded. Total MF-ERG recording time was eight minutes, a break was given after each 30 seconds of recording to facilitate good fixation. Data from recording sessions were obtained from each subject and averaged. The first –order MF-ERG namely the p amplitude, p latency were analyzed.

For each wave form the amplitude and latency of the first positive peak p were determined. The p amplitude was measured from the most negative through of the waveform to the most positive peak of MF-ERG waveform. The p latency is defined as the time taken from the onset of the stimulus to reach the most positive peak of the wave form. First order response is derived from the average retinal response to focal flash and reflects activities from the outer to middle retinal layers especially bipolar cells.

To analyze the 61-MF-ERG responses from each eye, three grouping configuration were used, all traces, rings and quadrants. All traces grouping was a single waveform grouping response from stimulus hexagons. The five rings grouping was five wave form grouping responses from five concentric rings. Rings1 is the most central hexagons with radius of about 0.5mm. Rings 2, 3, 4 and 5 were responses of increasingly eccentric annuli of stimulus.

The four quadrants grouping was four-wave form grouping response from superonasal, superotemporal, inferotemporal and inferonasal.

Fluorescein angiography (FA):

FA was done using (Topcon Corporation, 2000.TRC, 50ll, and japan)

Statistical analysis:

Data were analyzed using statistical package for social science (SPSS.15) for windows evaluation version. One way analysis of variance (ANOVA) was used to determine the statistical significance of the ERG changes in eyes with the stage of diabetic retinopathy. $P<0.05$ was taken to represent a significant difference. Spearman's correlation coefficient was used to calculate correlation.

Receiver operator characteristic (ROC) curves were constructed describing sensitivity and specificity of abnormal values for the control group versus patients, with optimal cut-off points chosen among normal and abnormal responses. The area under the curve (AUC) was used to compare the ROC curves. The comparison between AUCs was made according to the method reported by DeLong et al [15]. The sensitivity and specificity of the multifocal- ERG were compared to that of the full-field ERG.

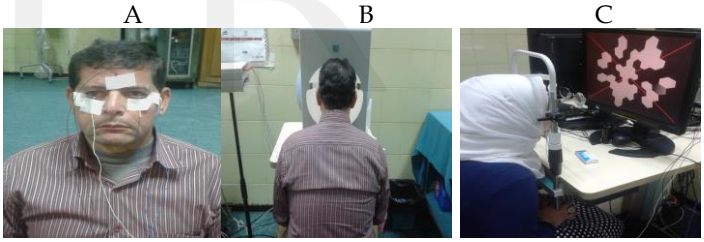

Fig1. (A) Patient with ERG connection; (B) patient on Ganzfeld stimulator; and (C) patient in front of MF-ERG.

3 Results:

The study included 30 subjects: ten were normal and twenty had diabetic retinopathy. Age and sex were included in table1.Norml control subject were free from any systemic diseases. Full field electroretinogram and multifocal electroretinogram were recorded and analyzed from both eyes of each subject.

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TABLE1

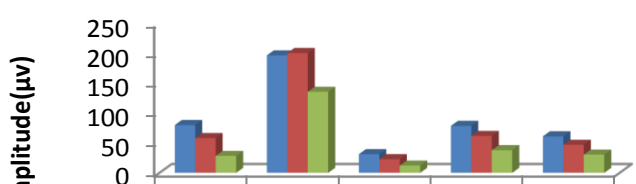
THE CLINICAL DATA OF SUBJECT

Group	*Number of subjects*	*Number of eyes*	*Sex*		*Age*
			male	*female*	
Group1 (control)	*10*	*20 eyes*	*3*	*7*	*43-58*
Group (diabetic retinopathy)	*20*	*40*	*9*	*11*	*45-60*
Non- Proliferativ	*10*	*20*			
Proliferative	*10*	*20*			

Standard (Fullfield) ERG

Scotopic and photopic recordings of F-ERG responses from normal, non-proliferative and proliferative diabetic retinopathy eyes were summarized in table2, 3(fig8) and in histograms in fig 2and 3Full field responses were significantly different among the three groups.

The implicit times of photopic a-waves and 30 HZ flicker showed no significant differences among the three groups (P=0.65, P=0.76) respectively. In non-proliferative diabetic retinopathy, there was a small alteration in the scotopic and photopic a- and b-waves amplitudes. In proliferative diabetic retinopathy which is more advanced stage the a- and b-waves and 30 HZ Flicker amplitudes are decreased. Abnormalities in the oscillatory potentials (OPs) had been demonstrated in the presence of normal a-and b-wave amplitudes. Progressive reduction in OP and 30 HZ flickers amplitudes as retinopathy increases in severity. The latencies were significantly prolonged in the two stages of diabetic retinopathy compared to normal subjects. There were significant differences in b/a ratio between eyes of normal control subjects and diabetic patients. The ratio tended to increase as the diabetic retinopathy progressed.



	rod response	standard combined(b-wave)	oscillatory potential	photopic response(b - wave)	30 HZ flicker
Normal	79.98	196.55	31.23	78.6	61.13
Non-proliferative	58.14	200.44	22.79	61.8	47
proliferative	28.41	135.56	12.19	37.85	30.49

Fig2: Amplitudes of full field ERG among groups

MF-ERG

The results from five rings and four quadrants of MF-ERG recordings of normal and diabetic eyes were summarized in Tables 4-5 and in histograms (fig 4-7).There was statistically significant difference between normal, non-proliferative and proliferative diabetic retinopathy. The mean p amplitudes of all trace grouping decreased (Figs 9).In patients with clinically apparent diabetic retinopathy, the latency of first positive peak was significantly increased ($P \leq 0.05$). The amplitudes of first order component were also significantly reduced. First order wave forms are known to vary mainly as a function of eccentricity. Hence, responses were averaged over concentric rings around the fovea for more accurate comparison of these parameters. The results in Table 4 and 5 held true for all eccentricities and also when first order responses were averaged over retinal quadrants (Figs. 4 – 6).The amplitudes of the MF-ERG in eyes with diabetic retinopathy were reduced relative to normal. Both normal and abnormal regions within eyes with diabetic retinopathy produced MF-ERG that was delayed relative to normal. The increased local severity of retinopathy was associated with increased delay of implicit time. No association between local MF-ERG amplitude and retinopathy grade was apparent.

TABLE 2

AMPLITUDES IN (µV) OF FULL FIELD ERG AMONG GROUP

	Normal	*Non proliferative*	*Proliferative*	*p-value*
Rod response	79.98±29.032	58.14±30.31	28.41±20.55	0.00
Standard combined a-wave	99.48±24.14	84.29±40.6	43.69±42.57	0.000
b-wave	196.55±47.24	200.44±54.77	135.56±70	0.004
b/a ratio	1.97±0.28	2.65±0.81	3.42±0.92	0.000
Oscillatory .potential	31.23±9.81	22.79±11.53	12.19±7.5	0.00
Photopic response a-wave	20.9±8.9	15.04±5.36	13.01±9.2	0.0014
b-wave	78.6±41.9	61.8±19.86	37.85±19.28	0.001
30 HZ flicker	61.13±30.6	47±11.22	30.49±13.7	**0.001**

There was statistically significant difference among groups except a-wave of photopic response and 30HZ flicker

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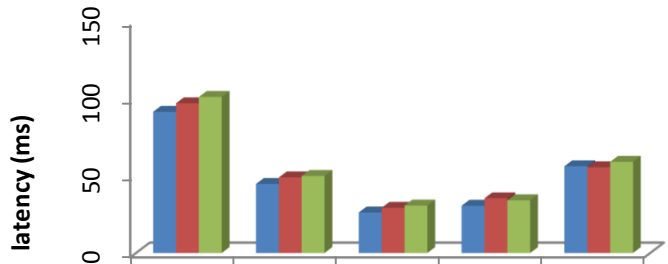
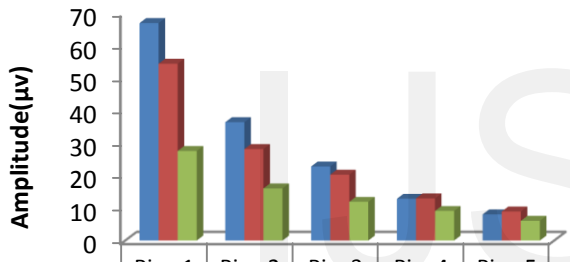


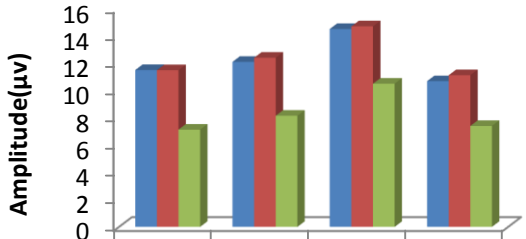
	rod response	standard combined(b-wave)	oscillatory potential	photopic response (b -wave)	30 HZ flicker
Normal	91.4	44.7	26.25	30.6	56.15
Non-proliferative	96.9	48.9	29.27	35.4	55.5
Proliferative	101	49.85	30.71	34.14	59

Fig 3: Latencies of Full field ERG among groups



	Ring 1	Ring 2	Ring3	Ring4	Ring 5
Normal	66.84	36.37	22.75	12.83	8.04
Non-proliferative	54.35	28.12	20.24	12.95	8.91
Proliferative	27.54	16.02	11.89	9.087	6.05

Fig 4: Mean (N1-P1) amplitudes for five rings of retina



	Superotemporal	Inferotemporal	Inferonasal	Superotemporal
Normal	11.5	12.1	14.51	10.68
Non-proliferative	11.48	12.4	14.7	11.12
Proliferative	7.13	8.15	10.5	7.4

Fig 5: Mean (N1-P1) amplitudes for four quadrants of retina

TABLE 3

LATENCIES IN (MS) OF FULL FIELD ERG AMONG GROUPS

	Normal	*Non proliferative*	*Proliferative*	*p-value*
Rod response	91.4 ± 7.61	96.9 ± 8.9	101 ± 10.91	0.01
Standard combined a-wave	22.45 ± 1.32	24.61 ± 1.91	23.28 ± 3.71	0.025
b-wave	44.7 ± 2.22	48.9 ± 4.28	49.85 ± 4.5	0.00
Oscillatory potential	26.25 ± 2.17	29.27 ± 3.08	30.71 ± 3.29	0.00
Photopic response a-wave	16.9 ± 5	18 ± 4.4	19 ± 7.32	0. 65
b-wave	30.6±1.19	35.4 ± 2.85	34.14 ± 5.36	0.00
30 HZ flicker	56.15±13.66	55.5±14.22	59 ± 13.32	**0.76**

TABLE 4

MEAN (N1-P1) AMPLITUDES IN (µV) FOR FIVE RINGS AND FOUR QUADRANTS OF RETINA

	Normal	*Non proliferative*	*Proliferative*	*p-value*
Ring1	66.84±34.15	54.35±31.69	27.54±22.68	0.002
Ring2	36.37±16.14	28.12±14.9	16.018±14.46	0.002
Ring3	22.75±9.14	20.24±8.9	11.89±5.19	0.001
Ring4	12.83±5.47	12.95±4.4	9.087±4.34	0.051
Ring5	8.036±4.31	8.91±3.72	6.0521±3.37	0.012
Supero temporal	11.5±8.6	11.48±5.43	7.13±4.34	0.12
Infero temporal	12.1±6.8	12.4±5.23	8.15±5.7	0.1
Ifero nasal	14.51±4.88	14.7±7.16	10.5±5.38	0.09
Supero nasal	10.68±5.99	11.12±5.28	7.4±4.69	**0.1**

- *There was statistically significant difference among groups in all rings of retina.*
- *There was no statistically significant difference among groups in all quadrants of retina.*

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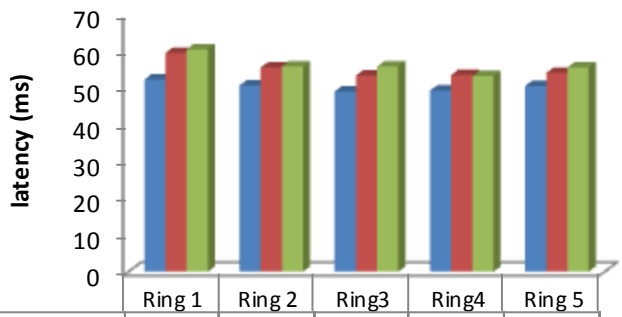
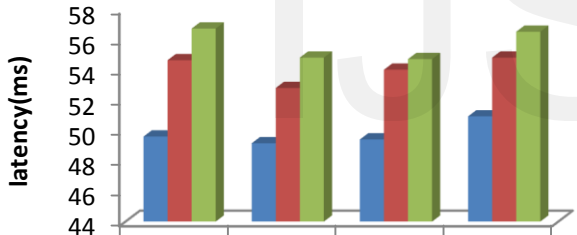


	Ring 1	Ring 2	Ring3	Ring4	Ring 5
Normal	52.31	50.68	49.07	49.3	50.54
Non-proliferative	59.54	55.56	53.41	53.5	54.1
Proliferative	60.5	55.86	55.82	53.21	55.6

Fig 6: Mean latencies for five rings of retina



	Superotemporal	Inferotemporal	Inferonasal	Superotemporal
Normal	49.6	49.16	49.41	50.93
Non-proliferative	54.63	52.8	54	54.8
proliferative	56.71	54.8	54.7	56.5

Fig 7: Mean latencies for four quadrants of retina

Correlation of MF-ERG and standard ERG:

Most of eyes affected with retinopathy had abnormal MF-ERG response compared with full field ERG response. Because MF-ERG is thought pre-dominantly to reflect cone function [16]. In eyes with non- proliferative diabetic retinopathy a significant correlation was found between ring 5 amplitude and the standard combined response amplitude (r = 0.5) and also strongly correlated with the cone response (r = 0.7) and 30-Hz flicker(r = 0.77) also a mild correlation found between ring 4 amplitude and cone response (r =o.61) and 30 HZ flicker(r = 0.68). (table6)

TABLE 5

MEAN LATENCIES IN (MS) FOR FIVE RINGS AND FOUR QUADRANTS OF RETINA

	Normal	*Non - proliferative*	*Proliferative*	*p-value*
Ring 1	52.31±8.24	59.54±6.47	60.5±12.84	0.017
Ring 2	50.68±3.98	55.56±4.25	55.86±4.1	0.00
Ring 3	49.07±3.13	53.41±2.94	55.82±3.96	0.00
Ring 4	49.3±3.36	53.5±3.5	53.21±5.56	0.005
Ring 5	50.54±3.00	54.1±3.6	55.6±2.87	0.00
superotemporal	49.6±4.23	54.63±3.91	56.71±3.65	0.00
Inferotemporal	49.16±3.54	52.8±3.79	54.8±4.21	0.00
Iferonasal	49.41±2.87	54±3.28	54.7±2.47	0.001
Superonasal	50.93±4.2	54.8±4.48	56.5±3.5	**0.001**

There was statistically significant difference among groups

Similar correlations were also seen between the implicit times of the cone (r=0.84 for ring 5) and standard combined response (r = 0.88 for ring 5) in the Ganzfeld and MF-ERG. There were no correlation between the 30 HZ flicker and the MF-ERG response ring averages.

In proliferative diabetic retinopathy (advanced case) (table7) good correlation was found between standard combined response and ring 5 (r = 0.52) and also with ring 3 (r = 0.5). Mild correlations were also seen between the implicit times of 30HZ and ring1.

Sensitivity and specifity:

Sensitivity and specificity are terms used to evaluate a clinical test.

The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease.

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives + False negatives}}$$

The specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease.

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives + False positives}}$$

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TABLE 6: CORRELATION COEFFICIENTS (SPEARMAN'S RHO [RS]) FOR ELECTRORETINOGRAM AMPLITUDES (WHITE TRIANGLE) AND LATENCIES (GREY TRIANGLE) IN CASE OF NON - PROLIFERATIVE DIABETIC RETINOPATHY

	MfERG Ring1	MfERG Ring2	MfERG Ring3	MfERG Ring4	MfERG Ring5	Rod response	Standard combined b-wave	Op	Cone response	30Hz
MfERG Ring1		0.80	0.63	0.026	0.13	-0.14	-0.043	0.23	0.15	-0.031
MfERG Ring2	0.21		0.82	0.43	0.45	0.037	0.095	0.26	0.27	0.24
MfERG Ring3	0.44	0.58		0.67	0.67	0.086	0.097	0.34	0.40	0.44
MfERG Ring4	0.30	0.44	0.77		0.90	0.35	0.46	0.44	0.61	0.68
MfERG Ring5	0.38	0.48	0.82	0.94		0.40	0.5	0.42	0.70	0.77
Rod response	0.12	-0.17	0.25	0.26	0.20		0.68	0.49	0.56	0.62
Standard combined-b-wave	0.46	0.51	0.69	0.85	0.88	0.15		0.83	0.63	0.63
op	0.20	0.50	0.64	0.89	0.84	0.24	0.85		0.60	0.50
Cone response	0.45	0.51	0.75	0.85	0.84	0.079	0.79	0.88		0.77
30Hz	0.054	-0.072	-0.14	-0.47	-0.41	0.22	-0.43	-0.26	-0.21	

TABLE 7: CORRELATION COEFFICIENTS (SPEARMAN'S RHO [RS]) FOR ELECTRORETINOGRAM AMPLITUDES (WHITE TRIANGLE) AND LATENCIES (GREY TRIANGLE) IN CASE OF PROLIFERATIVE DIABETIC RETINOPATHY

	MfERG Ring1	MfERG Ring2	MfERG Ring3	MfERG Ring4	MfERG Ring5	Rod response	Standard combined b-wave	op	Cone respon se	30Hz
MfERG Ring1		0.6	0.67	0.4	0.54	0.08	0.28	0.13	0.029	0.09
MfERG Ring2	0.22		0.73	0.53	0.57	0.02	0.30	0.01	0.029	-0.006
MfERG Ring3	0.1	0.65		0.7	0.69	0.29	0.50	0.30	0.18	0.17
MfERG Ring4	-0.18	0.43	0.55		0.68	0.29	0.48	0.29	0.44	0.39
MfERG Ring5	0.21	-0.088	0.52	0.22		0.34	0.52	0.32	0.44	0.42
Rod response	-0.29	-0.09	0.11	-0. 24	0.04		0.74	0.73	0.71	0.75
Standard combined-b-wave	-0.40	0.43	0.35	0.25	0.13	-0.034		0.87	0.68	0.72
Op	-0.65	-0.24	0.15	0.40	0.088	0.058	0.022		0.69	0.72
Cone response	0.053	0.23	0.27	0.094	0.38	-0.46	0.69	-0.088		0.94
30Hz	0.59	-0.007	0.18	-0.23	0.42	0.15	-0.20	-0.37	0.021	

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Receiver operator characteristic curves are a plot of false positive rate (1-specificity) of a test on the x-axis against its sensitivity on the y-axis for all possible cut-off points. The area under this curve (AUC) represents the overall accuracy of a test, with a value approaching 1.0 indicating a high sensitivity and specificity [17].

ROC Curves of Full-Field and multifocal ERGs:

The results from the 20 diabetic patients and from the 10 controls were used to construct ROC curves for the responses as shown in Figures 13and 14 curves from which the AUC was obtained(table8) . The proportion of eyes classified as abnormal or true positive rate (sensitivity) was plotted against the proportion of control eyes classified as abnormal or false Positive rate.

In early DR, the MF-ERG amplitude curves were always superior to the full-field amplitude curves.

The areas under the ROC curves ranged from 0.247 to0.543 with full field ERG and from 0.648 and 0.857with MF-ERG.(table8).As a result, the AUC of the MF-ERG was significantly larger than that of the full-field amplitude (Figure13) $P < 0.05$. As a result, the MF-ERG responses yielded the greatest sensitivity and specificity as seen in Table 8.The P1-N1 amplitude values of the rings 1, 2 and 3 presented the best area, sensitivity and specificity (Figures 13.b and Table 8).

In eyes with advanced DR, the ROC curves of the MF-ERGs and full-field ERGs were overlapped (Figure 14). The differences in the AUCs between the full-field and MF- ERG amplitude were not significant [18].

Sensitivity and Specificity of Full-Field and multifocal ERG

The sensitivity and specificity were obtained with the optimal cut-off values for the F-ERG and MF-ERG amplitude (Table 9). Because the likelihood ratio reveals the sensitivity/false positive rate, the highest likelihood ratio indicates high sensitivity and specificity.

In patients with NPDR, the sensitivities of the MF-ERG were significantly higher than those of the F-ERG ($P < 0.05$).

In PDR, the sensitivities of the MF-ERG were generally higher than those of the F-ERG.

TABLE 8

AREA UNDER THE CURVE OF THE F-ERG AND MF-ERG AMPLITUDES IN EARLY AND ADVANCED STAGES

	AUC of NPDR (early stage)	AUC of PDR (advanced)
n= 20		
Full field ERG		
Rod response	0.247	0.753
Standard combined	0.543	0.457
Oscillatory- potential	0.261	0.739
Photopic response	0.368	0.632
30 HZ flicker	0.351	0.649
n=20		
Multifocal ERG		
Ring1	0.857	0.932
Ring2	0.857	0.813
Ring3	0.879	0.946
Ring4	0.696	0.861
Ring5	0.648	0.864

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TABLE 9
SENSITIVITY AND SPECIFICITY OF THE F-ERG AND MF-ERG AMPLITUDES TO DISCRIMINATE EYES WITH DIABETIC RETINOPATHY

	Sensitivity percentage	Specificity percentage	Cut-off values
NPDR (n= 20)			
Full field ERG			
Rod response	36	50	57.6
Standard combined	53	52	144
Oscillatory potential	42	34	25.4
Photopic response	34	44	67.4
30 HZ flicker	38	66	51
Multifocal ERG			
Ring1	58	64	45
Ring2	50	47	29
Ring3	62	62	17.5
Ring4	60	58	11
Ring5	54	58	8.66
PDR (n=20)			
Full field ERG			
Rod response	70	65	61
Standard combined	70	65	192
Oscillatory potential	71	66	25.4
Photopic response	60	58	64.5
30 HZ flicker	57	58	51
Multifocal ERG			
Ring1	92	76	50
Ring2	78	68	164
Ring3	92	75	23
Ring4	86	80	46.5
Ring5	85	88	41.5

4 DISCUSSION

Electroretinography is a diagnostic tool that is used to record the electrical responses of retinal tissues to light stimulation. ERG waves are the summation of multiple signals that originate from neural and non-neural cells in the retina. All responses from retinal cells are expressed and visualized as negative and positive electrical waves. Therefore, based on an analysis and comparison of these waves, we are able to evaluate retinal function and to recognize specific retinal lesions [19].

Under scotopic conditions, the wave is directly generated by bipolar cells [20]. Under photopic conditions, several types of neurons contribute to the generation of the response [21]. Ops are four to six low amplitude, high frequency wavelets superimposed on the ascending limb of ERG b-wave. The Ops are thought to result from feedback between the amacrine cells and the bipolar cell and/or feedback from ganglion cells to amacrine cells [22]. Full field Ganzfeld ERG in the current study showed reductions in scotopic and photopic responses in diabetic patients with retinopathy. Reduction in oscillatory potential amplitude and flicker response were also observed in those patients with delayed implicit times. Other ERG Studies of diabetic patients have reported inconsistent results. Arden et al.[23] found reduced ERG amplitudes of diabetic patients only in the presence of cotton wool spots and angiographic evidence of capillary non-perfusion whereas others, reported normal or even supernormal amplitudes in the flash ERG of diabetic patients with retinopathy. Wanger and Persson [24] could not find any flash ERG changes that could distinguish between the presence or absence of retinopathy in diabetic patients. Reduction in amplitudes of oscillatory potentials has also been reported in diabetic retinopathy. [25] However, others did not find any such changes.[26]Bresnick and Palta[27] and Hood and Birch[28] reported that Ops amplitudes correlate well with the severity of diabetic retinopathy. Whereas Chung et al. [29] and Satoh et al.[30] observed that photopic b-wave implicit times correlate well with severity of diabetic retinopathy.

In the current study there was statically significant correlation between oscillatory potential amplitudes and severity of diabetic retinopathy (R=0.64, P=0.014).

MF–ERG technique can measure and map retinal functions at more than 60 locations within 8 min and can distinguish separate responses of inner and outer retina. MF-ERG has been used to examine a large number of eye diseases including diabetes related retinal function. [31] Several reports have provided information regarding retinal dysfunction associated with DM. Investigators who have analyzed MF-ERG have reported abnormally reduced amplitudes and/or delayed implicit times in diabetic subjects[32]and in diabetic subjects without signs of retinopathy.[33,34]

.In the current study, there were reductions in the amplitudes of first order and delays in implicit times in patients with diabetic retinopathy. Farahvash and Mohammed Zadoh [35] reported that local ERG responses were significantly delayed and decreased in amplitude in patients with significant diabetic edema. Similarly, Fortune et al. [36] reported that implicit times were increased and amplitudes were mildly reduced. Green stein et al. [37] found that implicit times were significantly increased. Weiner et al. [38] have reported that mean amplitude of ERG was lower in eyes without diabetic edema compared to normal eyes and was even lower in eyes with edema.

The magnitude of MF-ERG implicit time delays was correlated with the severity of retinopathy. By contrast, response amplitude although reduced in eyes with retinopathy had no such correlation with the degree of retinopathy in this study.

The increased implicit time of local ERG responses were associated with increased severity of the local retinopathy signs.[36] Bearse et al. [39]and Han et al.[40] found no correlation between amplitude reduction and retinal abnormalities. ERG abnormalities can be present at a very early stage of disease while there are no visible changes in the fundus and before the onset of clinical symptoms.

The cause that amplitude of first order was normal in early diabetes is likely because the first order response components originate predominantly in the outer (cone photoreceptors) retina and/or middle retina (cone bipolar cells, Muller cells).

Sutter and Tran [41] showed that under photopic conditions, the decreased first order response component with eccentricity follows approximately that of retinal cone density. In addition, when all the focal responses of the multifocal cone ERG are averaged together, the response bears a strong similarity to the full field flash ERG. Flash ERG responses originate predominantly in the outer 70% of the retina. The reduced overall amplitudes and the delayed latencies in the first order component observed in diabetic patients with retinopathy may indicate some impairment of outer retinal function in diabetes.

MF-ERG amplitudes changes of first order component response were not associated with early retinopathy. One possible reason for the insensitivity of amplitude to diabetic dysfunction is that this measure has larger inter-subject variability than implicit time in normal subjects. This large inter-individual variability of amplitude diminishes the usefulness of this parameter for detection of local retinal abnormalities. In contrast, the variability in MF-ERG implicit times was very small, consistent with the findings of other MF-ERG studies.

Another consideration is that amplitude measures reflect the strength of the summed responses generated by retinal cells and may be significantly affected only at a later stage when the generators are severely damaged or cell loss occurs. Additionally it has been demonstrated that decreased stimuli contrast or luminance affect MF-ERG amplitude to much greater extent than implicit time. Thus it is possible that decreased effective stimulus contrast and/or luminance within patches of retinal abnormalities may be responsible for alteration of local MF-ERG amplitude in diabetic retinopathy.

Dolan et al. found good correlation between MF-ERG in central region and flicker amplitude [42]. In the current study, correlation was found between ring 5 amplitude and the standard combined response amplitude (r=0.5) and with the cone response (r=0.7) and 30-Hz flicker(r=0.77) in non-proliferative diabetic retinopathy. Similar correlations were also seen between the implicit times of the cone (r=0.84 for ring 5) and standard combined response (r=0.88 for ring 5) in the Ganzfeld and MF-ERG. We found no correlation between the 30 HZ flicker and the MF-ERG response ring averages.

In proliferative diabetic retinopathy, good correlation was found between mixed cone- rod response and ring5(r= 0.52) and also with ring3(r= 0.5). Mild correlations were also seen between the implicit times of 30HZ and ring1.

This study shows that more of MF-ERG responses from both the non-proliferative and proliferative diabetic retinopathy differ significantly from control than did full field ERG responses. The reason for this may be explained that :the MF-ERG response, is a result of multiple frequencies of stimulation as opposed to standard ERG wave form which is a single frequency of stimulation[43].Therefore it is likely that MF-ERG reflects more of non-linear processes in the retina .

MF-ERG is well suited to the study of diabetic retinopathy for several reasons: Firstly, diabetic retinopathy is a retinal disease with local lesion typically confined to the posterior pole where the MF-ERG techniques test local retinal function (the central 45) [30]; Secondly, diabetic retinopathy is largely caused by defects of retinal capillaries in the inner nuclear layer where the cell bodies of the bipolar cells, the primary generators of MF-ERG are located. Thus there is an anatomic basis for the detection of MF-ERG abnormality in diabetes.

MF-ERG provides very sensitive objective assessment of local retinal health in diabetes mellitus. MF-ERG implicit time is a sensitive measure of retinal function that can be used to monitor the progression of diabetic retinopathy. It is evident that, MF-ERG is abnormal very early in diabetic retinopathy.

MF-ERG can demonstrate local abnormalities while the abnormalities in F-ERG demonstrate the widespread nature of retinopathy.

In the current study, we compared abilities between F-ERG and MF-ERG in detecting DR eyes. Our results demonstrated that the AUCs and sensitivities were higher for the MF-ERG than for the F-ERG at the early and advanced stages of DR. The AUCs of the MF-ERG were better for identifying eyes with early DR than those of the F-ERG On the other hand; there was no significant difference in the AUCs between the MF-ERG and F-ERG in advanced DR. We selected the optimal cut-off value with the highest likelihood ratio which maximally reduces false positive cases. As a result MF-ERG proved to be more sensitive and specific than standard full field ERG in detecting DR. It was shown subsequently to be able to detect cases of retinopathy before full field electroretinographic testing showed abnormalities.

5 Conclusion

MF-ERG is more susceptible to ocular changes of DR than standard ERG due to the multiple frequencies of stimulation used to record MF-ERG response. MF-ERG could be a sensitive indicator of underlying disease affecting the retina in eyes with DR. MF-ERG has the diagnostic ability with higher sensitivity in detecting early DR than F-ERG.

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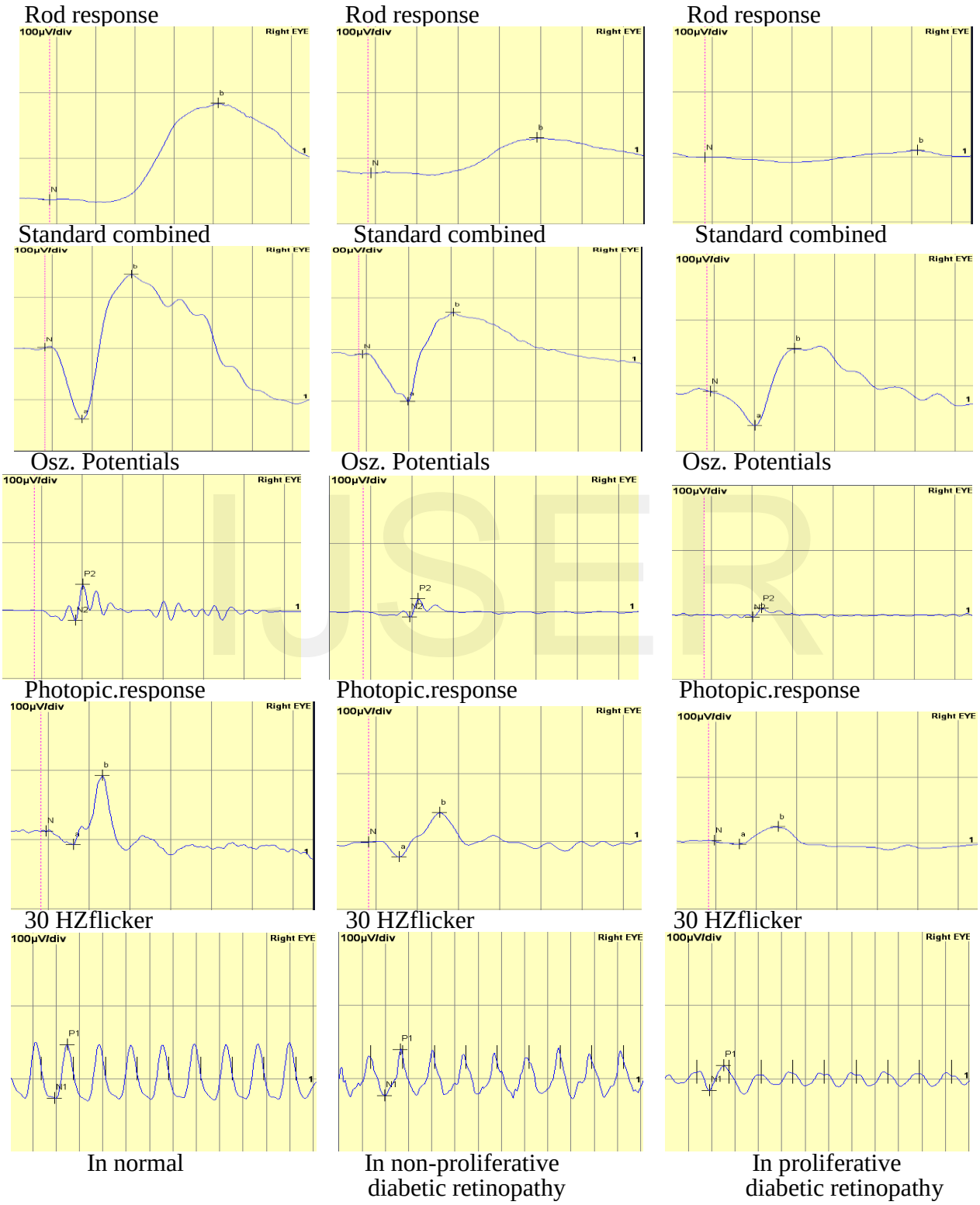

Fig8. Full field ERG among groups

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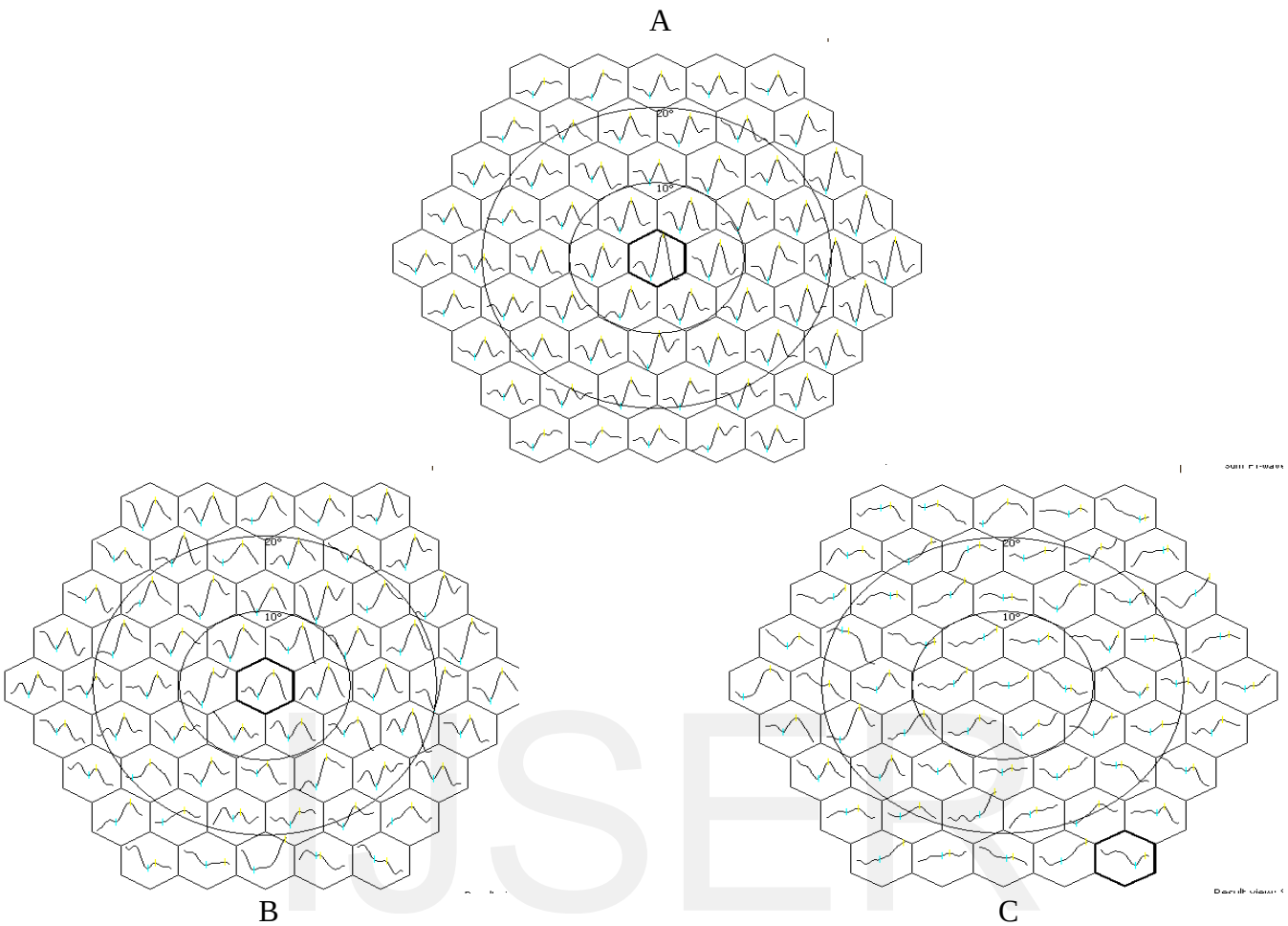

Fig9: First order component of MF-ERG among groups. (A) MF-ERG in control group;(B) MF-ERG in diabetic eyes with non-proliferative retinopathy in which there is mild reduction of amplitude & delay in latency; (C) MF-ERG in proliferative retinopathy in which no apparent peak and through.

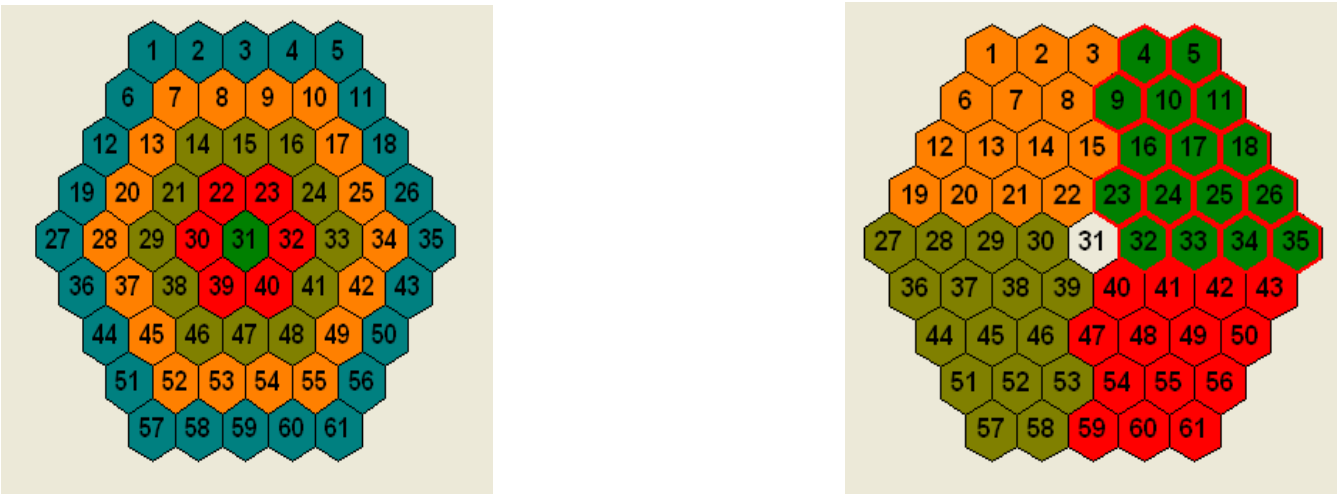

Fig10: MF-ERG over rings and quadrants in normal subjects

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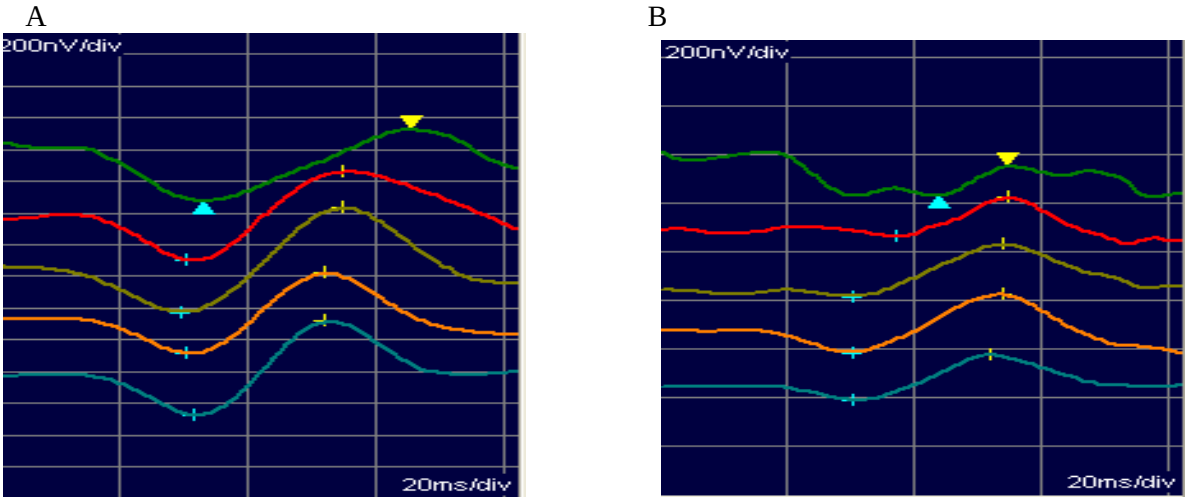

Fig 11: MF-ERG over rings in DR.: (A) With NPDR and (B) With PDR

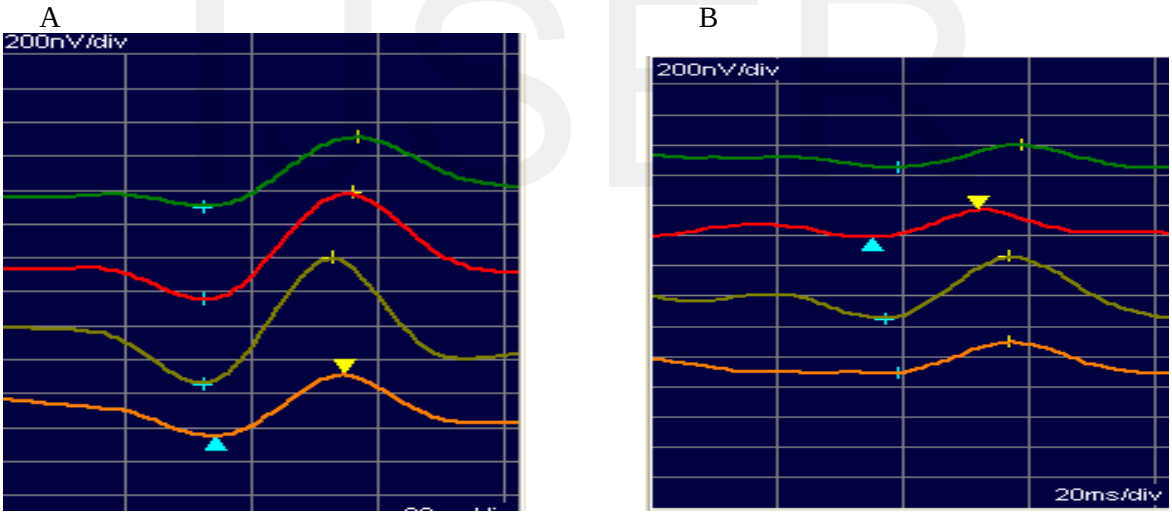

FIG12: MF-ERG over Quadrants in DR.: (A) With NPDR and (B) With PDR

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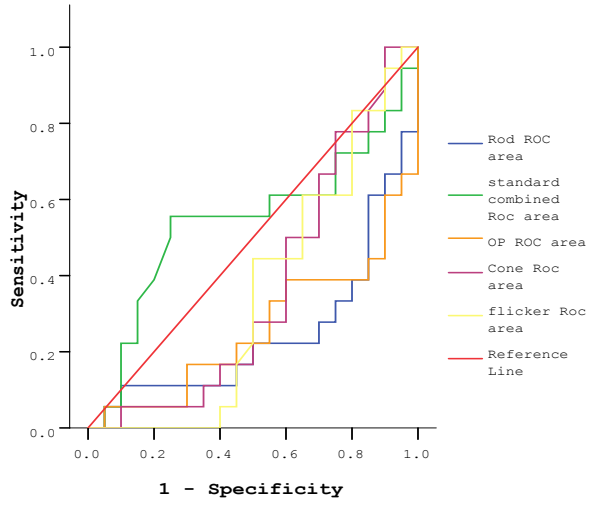

Fig 13:a. ROC curves of full field ERG. ROC curves describing sensitivity and specificity of full field ERG wave amplitude values for the control group versus non proliferative diabetic retinopathy patients. The areas under the ROC curves range from 0.247 to0.543.
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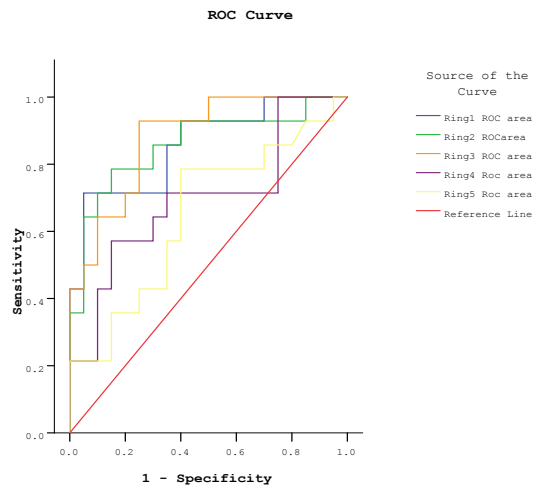

Fig 13: b. ROC curves of MF-ERG (P1-N1). ROC curves describing sensitivity and specificity of N1-P1 wave amplitude values for the control group versus non-proliferative diabetic retinopathy areas under the ROC curves range from 0.648 to 0.857

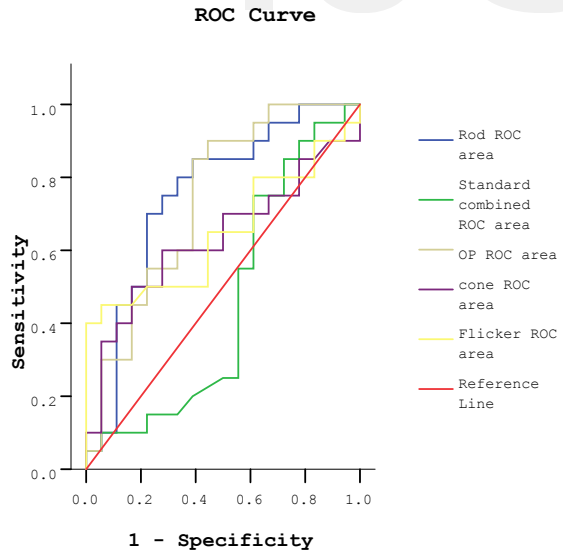

Fig 14:a. ROC curves of full field ERG. ROC curves describing sensitivity and specificity of full field ERG wave amplitude values for the control group versus proliferative diabetic retinopathy patients. The areas under the ROC curves range from 0.649 to0.753
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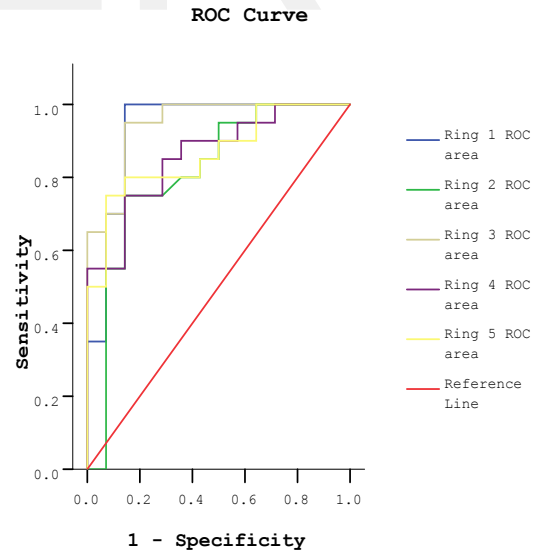

Fig 14: b. ROC curves of MF-ERG (P1-N1). ROC curves describing sensitivity and specificity of N1-P1 wave amplitude values for the control group versus proliferative diabetic retinopathy areas under the ROC curves range from 0.864 to 0.932

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