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ORIGINAL RESEARCH ARTICLE

# Multifocal electroretinography in subjects with age-related macular degeneration

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**Abstract**
*Purpose* To evaluate retinal function objectively in subjects with different stages of age-related macular degeneration (AMD) using multifocal electroretinography (mfERG) and compare it with age-matched control group.
*Methods* A total of 42 subjects with AMD and 37 age-matched healthy control group aged over 55 years were included in this prospective study. mfERG test was performed to all subjects. Average values in concentric ring analysis in four rings (ring 1, from 0° to 5° of eccentricity relative to fixation; ring 2, from 5° to 10°; ring 3, from 10° to 15°; ring 4, over 15°) and in quadrant analysis (superior nasal quadrant, superior temporal quadrant, inferior nasal quadrant and inferior temporal quadrant) were recorded. Test results were evaluated by one-way ANOVA test and independent samples *t* test.
*Results* In mfERG concentric ring analysis, N1 amplitude, P1 amplitude and N2 amplitude were found to be lower and N1 implicit time, P1 implicit time and N2 implicit time were found to be delayed in subjects with AMD compared to control group. In quadrant analysis, N1, P1 and N2 amplitude was lower in all quadrants, whereas N1 implicit time was normal and P1 and N2 implicit times were prolonged in subjects with AMD.
*Conclusion* mfERG is a useful test in evaluating retinal function in subjects with AMD. AMD affects both photoreceptors and inner retinal function at late stages.

**Keywords** Age-related macular degeneration · Multifocal ERG · Photoreceptors · Retina

## Introduction

Age-related macular degeneration (AMD) is one of the leading causes of irreversible vision loss among patients aged over 55 years. Its etiopathogenesis has not been fully understood yet but abnormalities are seen in photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris [1]. The first morphologic change in AMD is the extracellular material deposition under RPE. Besides, accumulation of phospholipids in the inner collagen layer of Bruch's membrane leads to a failure in retinal function [2]. AMD has three stages: early, intermediate and late stage. Early stage of AMD includes medium drusen $\geq$63 and $\leq$125 μm and no pigmentary abnormalities; intermediate stage of AMD includes large drusen $\geq$125 μm and/or any pigmentary abnormalities; and late stage of AMD includes any geographic atrophy or neovascular AMD [3]. Progression to late AMD may be

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related with severe loss of visual acuity. So, identifying patients at increased risk of late AMD is very important.

Full-field electroretinogram (ERG) is a standard clinical test for evaluating global retinal function. A more detailed topographic evaluation of retinal activity can be done using multifocal ERG. Multifocal ERG changes have been shown in different macular disease including AMD [4–7]. In this study, we aimed to evaluate retinal function objectively by multifocal ERG responses in subjects with early/intermediate AMD and subjects with late AMD and to compare these results with age-matched control group.

## Materials and methods

This prospective study was conducted in Afyon Kocatepe University department of Ophthalmology, and the study was approved by the institutional ethics committee. Written informed consent was taken from all subjects. Forty-two subjects aged over 55 years with the diagnosis of AMD were included in the study. Thirty-seven age-matched healthy subjects served as control group. Right eyes were included in the study. If there was an ocular pathology such as cataract that could affect multifocal ERG recordings, the left eye was included in the study. Subjects with any history of systemic disease such as hypertension or diabetes mellitus, using systemic medication such as hydroxychloroquine or vigabatrin, or any ocular disease such as high myopia or glaucoma that could influence multifocal ERG responses were excluded from the study.

Routine ophthalmic examination was performed. Visual acuity was measured using ETDRS chart. Biomicroscopic examination was performed. After measurement of intraocular pressure using applanation tonometer, dilated fundus examination was performed to all subjects. Diagnosis of AMD was performed according to the clinical classification report of macular research classification committee [3]. Persons with small drusen (<63 μm) were considered to have normal aging changes. Persons with medium drusen (63–125 μm), but without pigmentary abnormalities, were considered to have early AMD. Persons with large drusen (>125 μm) or with pigmentary abnormalities associated with at least medium drusen were considered to have intermediate AMD. Persons with lesions associated with neovascular AMD or geographic atrophy were considered to have late AMD. None of the subjects in our control group had any age-related retinal changes. Though increasing amounts of large drusen and pigmentary abnormalities seem to be important in progression to late stage of AMD, as severe visual loss is known to be associated with late AMD, we classified subjects into two groups: subjects with early/intermediate AMD (15 subjects) and subjects with late AMD (27 subjects).

Multifocal ERG recordings were performed using Metrovision Monelec 2 (Metrovision, France). The pupils were fully dilated before the test using tropicamide 1.0 % (Tropamid fort %1, Bilim, Turkey). The recording was performed using an active ERG jet corneal contact lens electrode where the electrode was centered with the pupil. Before the reference electrode and neutral electrode were placed on the skin, the skin was prepared with a slightly abrasive paste and cleaned with alcohol so as to remove the superficial dry layer and oily layer, which are thought to be poor electric conductor. Then, the neutral electrode was attached centrally on the patient's forehead slightly above the supraorbital rims. The reference electrode was placed near to the outer canthus. The electrodes were connected with a junctional box, from which the signals were delivered to additional recording components for amplification and display. The fellow eye was occluded. The test was performed from 33 cm distance, and refractive error was corrected for 33 cm in all subjects using large field refractive lenses. The subject was installed on a chin rest, and fixation was controlled using a built-in near infrared camera during the test. MERG61H test (mfERG with hexagons of equal dimensions) was performed in accordance with ISCEV guidelines, and responses from 61 retinal locations were recorded in approximately 5 min [8]. The stimulative visual angles subtended ±30° horizontally and ±23° vertically. Electrical noise that shows the quality of the recorded signal was recorded, and test results with a noise level of >5 μv were excluded from the study. Number of valid and rejected responses was recorded, and if rejected responses were >20 % of total responses, the test was excluded from the study. Maps of local and global responses were obtained. Group averages that provide the characteristics of average responses over a group of up to four rings were computed by the program. These four rings were: ring 1, from 0° to 5° of eccentricity relative to fixation; ring 2, from 5° to 10°; ring 3, from 10° to 15°; and ring 4, over 15°. Analysis of group averages in

superior nasal quadrant, superior temporal quadrant, inferior nasal quadrant and inferior temporal quadrant was also performed. Mean amplitude (nV, nanovolt) and implicit time (ms) was recorded. N/Np, P/Pp and P/N indices were calculated. N/Np index is the ratio between N1 amplitude of a given ring and N1 amplitude of the periphery; P/Pp index is the ratio between P1 amplitude of a given ring and P1 amplitude of the periphery; P/N index is the ratio between the amplitude of P1 and the amplitude of N1 for a given ring.

Data were expressed as mean ± SD. Statistical analysis was performed using SPSS 20.0. Distribution of normality was evaluated by Kolmogorov–Smirnov test. The results were compared using one-way ANOVA test followed by Scheffe post hoc test and independent samples $t$ test. $P$ values <0.05 were considered to be significant.

## Results

Mean age was 64.05 ± 8.4 years in control group and 67.76 ± 1.4 years in subjects with AMD ($p = 0.060$). In control group, 19 (51.4 %) of the subjects were female and 18 (48.6 %) were male, whereas in subjects with AMD, 20 (47.6 %) of the subjects were female and 22 (52.4 %) were male ($p = 0.46$). Fifteen subjects with AMD were classified as early/intermediate AMD, whereas 27 subjects were classified as late AMD. Nineteen subjects (3 normal subjects, 9 subjects with early/intermediate AMD and 7 subjects with late AMD) were excluded from the study because of high electrical noise and poor quality of mfERG recordings.

Mean visual acuity was 0.00 ± 0.0 logMAR in control group, 0.22 ± 0.2 logMAR in subjects with early/intermediate AMD and 0.67 ± 0.4 logMAR in subjects with late AMD ($p < 0.001$). The difference in visual acuity was significant between control group and subjects with early/intermediate AMD, between control group and subjects with late AMD, and between subjects with early/intermediate AMD and late AMD ($p = 0.001$, $p < 0.001$, $p < 0.001$, respectively).

Mean electrical noise for mfERG recordings was 2.3 ± 0.6 μV in control group, 2.3 ± 0.5 μV in subjects with early/intermediate AMD and 2.4 ± 0.4 μV in subjects with late AMD ($p = 0.66$). Multifocal waves of a control subject, early/intermediate AMD subject and late AMD subject are shown in Figs. 1, 2 and 3. When we evaluated subjects with AMD without dividing into subgroups, ring analysis results showed a significant decrease in amplitudes of N1, P1 and N2 in subjects with AMD and the implicit times were prolonged in especially P1 and N2. In control group, mean amplitude of N1 was 47.2 ± 17.2 nV in central 5°, 31.6 ± 8.2 nV in 10°, 23.1 ± 4.9 nV in 15° and 18.3 ± 3.7 nV in 25°, whereas in subjects with AMD, mean amplitude of N1 was 28.8 ± 18.7 nV in central 5°, 20.8 ± 8.9 nV in 10°, 15.5 ± 5.9 nV in 15° and 12.1 ± 5.7 nV in 25° (for all measurements $p < 0.001$). In control group, mean implicit time of N1 was 26.3 ± 5.3 ms in central 5°, 25.3 ± 4.9 ms in 10°, 25.3 ± 4.8 ms in 15° and 25.7 ± 4.9 ms in 25°, whereas in subjects with AMD, mean implicit time of N1 was 27.7 ± 3.8 ms in central 5°, 27.4 ± 2.7 ms in 10°, 26.6 ± 2.4 ms in 15° and 26.6 ± 2.4 ms in 25° ($p = 0.19$, $p = 0.017$, $p = 0.12$, $p = 0.24$, respectively). In control group, mean amplitude of P1 was 92.3 ± 31.7 nV in central 5°, 60.9 ± 15.1 nV in 10°, 42.7 ± 9.2 nV in 15° and 32.7 ± 6.7 nV in 25°, whereas in subjects with AMD, mean amplitude of P1 was 50.9 ± 36.1 nV in central 5°, 38.1 ± 18.5 nV in 10°, 31.1 ± 12.3 nV in 15° and 23.3 ± 9.4 nV in 25° (for all measurements $p < 0.001$). In control group, mean implicit time of P1 was 46.6 ± 5.5 ms in central 5°, 43.9 ± 5.1 ms in 10°, 43.5 ± 4.9 ms in 15° and 43.7 ± 4.9 ms in 25°, whereas in subjects with AMD, mean implicit time of P1 was 49.0 ± 5.0 ms in central 5°, 47.1 ± 3.7 ms in 10°, 46.0 ± 3.3 ms in 15° and 45.8 ± 3.3 ms in 25° ($p = 0.047$, $p = 0.002$, $p = 0.009$, $p = 0.021$, respectively). In control group, mean amplitude of N2 was 82.3 ± 30.8 nV in central 5°, 49.3 ± 14.8 nV in 10°, 33.7 ± 9.9 nV in 15° and 25.3 ± 7.3 nV in 25°, whereas in subjects with AMD, mean amplitude of N2 was 40.5 ± 33.3 nV in central 5°, 30.7 ± 17.1 nV in 10°, 24.5 ± 10.5 nV in 15° and 17.9 ± 8.0 nV in 25° (for all measurements $p < 0.001$). In control group, mean implicit time of N2 was 66.3 ± 5.9 ms in central 5°, 62.3 ± 5.9 ms in 10°, 61.2 ± 5.1 ms in 15° and 60.8 ± 4.7 ms in 25°, whereas in subjects with AMD, mean implicit time of N2 was 71.0 ± 7.0 ms in central 5°, 68.6 ± 8.0 ms in 10°, 63.4 ± 11.3 ms in 15° and 65.1 ± 8.6 ms in 25° ($p = 0.003$, $p < 0.001$, $p = 0.30$, $p = 0.013$, respectively).

Subgroup analysis results are given in Table 1. The decrease in N1, P1 and N2 amplitudes was significant

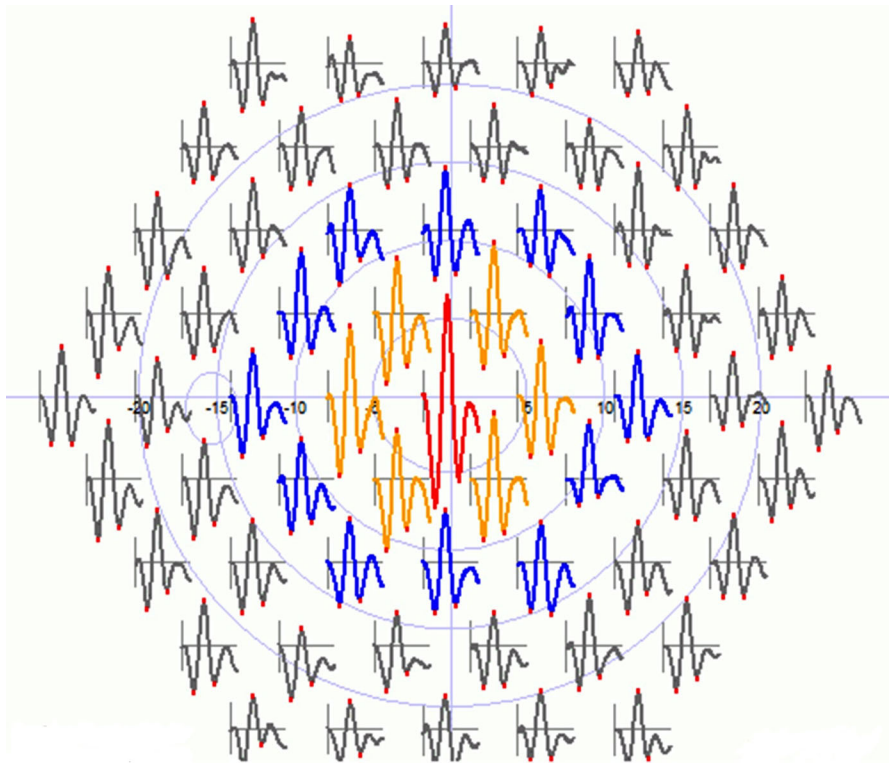


**Fig. 1** mfERG responses from a normal subject

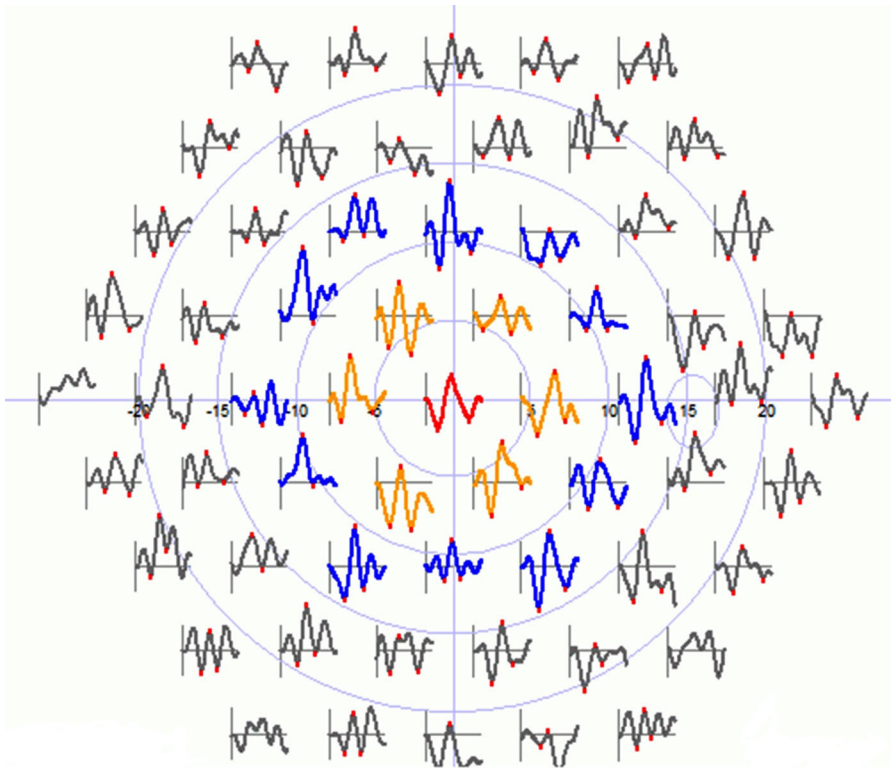


**Fig. 2** mfERG responses from a subject with early/intermediate age-related macular degeneration

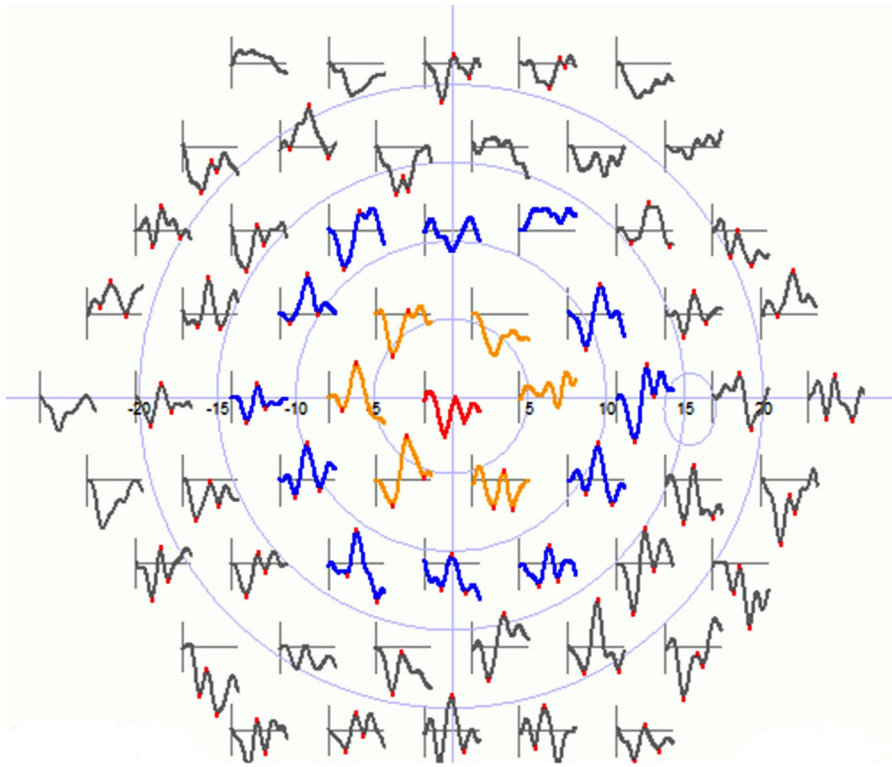


**Fig. 3** mfERG responses from a subject with late age-related macular degeneration

in both early/intermediate AMD and late AMD compared to control group, whereas we could find no significant difference between subjects with early/intermediate AMD and late AMD. Implicit times of N1 did not show any significant difference between groups. Implicit times of P1 were not affected in subjects with early/intermediate AMD compared to control group, whereas in subjects with late AMD, implicit time of P1 was prolonged in central 5°–15° (ring 2 and ring 3) compared to control group. Implicit times of P1 did not show any significant change between subjects with early/intermediate AMD and subjects with late AMD. In subjects with early/intermediate AMD, implicit time of N2 was found to be prolonged in ring 4 compared to control group, whereas in subjects with late AMD, implicit time of N2 was prolonged in ring 1, ring 2 and ring 4 compared to control group. Implicit times of N2 did not show a significant change between subjects with early/intermediate AMD and late AMD.

In quadrant analysis, amplitude of N1, P1 and N2 was significantly lower in subjects with AMD compared to control group (in all quadrants $p < 0.001$). Implicit time of N1 did not show any significant change between subjects with AMD and control group (for superior nasal quadrant, $p = 0.14$; for superior temporal quadrant, $p = 0.16$; for inferior nasal quadrant $p = 0.059$; for inferior temporal quadrant, $p = 0.22$). Implicit time for P1 was prolonged in subjects with AMD in all quadrants except inferior temporal quadrant (for superior nasal quadrant, $p = 0.008$; for superior temporal quadrant, $p = 0.012$; for inferior nasal quadrant, $p = 0.008$; for inferior temporal quadrant, $p = 0.11$). Implicit time for N2 was also prolonged in all quadrants in subjects with AMD (for superior nasal quadrant, $p = 0.002$; for superior temporal quadrant, $p = 0.008$; for inferior nasal quadrant, $p = 0.036$; for inferior temporal quadrant, $p = 0.004$).

Table 2 shows the difference among groups in quadrant analysis. Amplitude of N1 and P1 was found to be significantly decreased in subjects with early/intermediate AMD and late AMD in all quadrants compared to control group, whereas there was no significant difference between subjects with early/intermediate AMD and late AMD. Amplitude of N2 was found to be significantly decreased in all quadrants except inferior temporal quadrant in subjects with early/intermediate AMD compared to control

**Table 1** Zone analysis results in groups

| Parameter | Control ($n = 37$) | Early/int AMD ($n = 15$) | Late AMD ($n = 27$) | $p$ |
|---|---|---|---|---|
| N1 amplitude—ring 1 | $47.2 \pm 17.2^{a}$ | $31.3 \pm 23.4^{b}$ | $27.4 \pm 15.8^{b}$ | <0.001 |
| N1 amplitude—ring 2 | $31.6 \pm 8.2^{a}$ | $20.5 \pm 11.0^{b}$ | $20.9 \pm 7.7^{b}$ | <0.001 |
| N1 amplitude—ring 3 | $23.1 \pm 4.9^{a}$ | $14.7 \pm 6.6^{b}$ | $15.9 \pm 5.6^{b}$ | <0.001 |
| N1 amplitude—ring 4 | $18.3 \pm 3.7^{a}$ | $11.8 \pm 6.1^{b}$ | $12.2 \pm 5.6^{b}$ | <0.001 |
| N1 implicit time—ring 1 | $26.3 \pm 5.3^{ns}$ | $25.6 \pm 3.7^{ns}$ | $28.8 \pm 3.5^{ns}$ | 0.042 |
| N1 implicit time—ring 2 | $25.3 \pm 4.9$ | $27.7 \pm 3.4$ | $27.3 \pm 2.3$ | 0.057 |
| N1 implicit time—ring 3 | $25.3 \pm 4.8$ | $26.5 \pm 3.1$ | $26.7 \pm 2.1$ | 0.31 |
| N1 implicit time—ring 4 | $25.7 \pm 4.9$ | $26.3 \pm 2.2$ | $26.8 \pm 2.3$ | 0.46 |
| P1 amplitude—ring 1 | $92.3 \pm 31.7^{a}$ | $59.2 \pm 48.2^{b}$ | $46.2 \pm 27.3^{b}$ | <0.001 |
| P1 amplitude—ring 2 | $60.9 \pm 15.1^{a}$ | $37.5 \pm 22.5^{b}$ | $38.5 \pm 16.2^{b}$ | <0.001 |
| P1 amplitude—ring 3 | $42.7 \pm 9.2^{a}$ | $30.0 \pm 15.2^{b}$ | $31.9 \pm 10.6^{b}$ | <0.001 |
| P1 amplitude—ring 4 | $32.7 \pm 6.7^{a}$ | $22.4 \pm 11.4^{b}$ | $23.9 \pm 8.3^{b}$ | <0.001 |
| P1 implicit time—ring 1 | $46.6 \pm 5.5$ | $47.9 \pm 5.8$ | $49.6 \pm 4.5$ | 0.086 |
| P1 implicit time—ring 2 | $43.9 \pm 5.1^{a}$ | $47.2 \pm 4.4^{a,b}$ | $47.1 \pm 3.4^{b}$ | 0.008 |
| P1 implicit time—ring 3 | $43.5 \pm 4.9^{a}$ | $45.7 \pm 3.8^{a,b}$ | $46.1 \pm 3.1^{b}$ | 0.032 |
| P1 implicit time—ring 4 | $43.7 \pm 4.9$ | $46.5 \pm 4.5$ | $45.5 \pm 2.5$ | 0.056 |
| N2 amplitude—ring 1 | $82.3 \pm 30.8^{a}$ | $51.1 \pm 46.2^{b}$ | $34.6 \pm 22.4^{b}$ | <0.001 |
| N2 amplitude—ring 2 | $49.3 \pm 14.8^{a}$ | $32.8 \pm 20.9^{b}$ | $29.6 \pm 15.0^{b}$ | <0.001 |
| N2 amplitude—ring 3 | $33.7 \pm 9.9^{a}$ | $24.4 \pm 13.0^{b}$ | $24.5 \pm 9.1^{b}$ | 0.001 |
| N2 amplitude—ring 4 | $25.3 \pm 7.3^{a}$ | $16.9 \pm 9.4^{b}$ | $18.5 \pm 7.1^{b}$ | 0.001 |
| N2 implicit time—ring 1 | $66.3 \pm 5.9^{a}$ | $70.2 \pm 8.6^{a,b}$ | $71.4 \pm 6.1^{b}$ | 0.010 |
| N2 implicit time—ring 2 | $62.3 \pm 5.9^{a}$ | $66.9 \pm 6.5^{a,b}$ | $69.6 \pm 8.8^{b}$ | 0.001 |
| N2 implicit time—ring 3 | $61.2 \pm 5.1$ | $66.2 \pm 7.6$ | $61.7 \pm 12.8$ | 0.19 |
| N2 implicit time—ring 4 | $60.8 \pm 4.7^{a}$ | $66.9 \pm 10.7^{b}$ | $64.0 \pm 7.1^{b}$ | 0.023 |

Data are expressed as mean ± SD; ring 1, from 0° to 5° of eccentricity relative to fixation; ring 2, from 5° to 10°; ring 3, from 10° to 15°; ring 4, over 15°; AMD, age-related macular degeneration; early/int, early/intermediate, ns, not significant; $p$, one-way ANOVA

Different superscript letters (a, b) represent a significant difference between groups according to Sheffe post hoc test. Values that are not significantly different have the same superscript letters

group, whereas it was found to be decreased in all quadrants in subjects with late AMD compared to control group. Amplitude of N2 showed no significant change between subjects with early/intermediate AMD and late AMD. Implicit time of N1 did not show any significant change between groups in all quadrants. Implicit time of P1 in superior temporal quadrant was prolonged in subjects with early/intermediate AMD compared to control group, whereas implicit time of P1 in quadrants did not show any significant change in subjects with late AMD compared to control group and subjects with early/intermediate AMD. Implicit time of N2 was prolonged in superior temporal and inferior temporal quadrants in subjects with early/intermediate AMD compared to control group, whereas it was prolonged in superior nasal, superior temporal and inferior temporal quadrants in subjects with late AMD compared to control group. The change was similar between subjects with early/intermediate AMD or late AMD for N2 implicit times in quadrants.

P/Pp index was found to be decreased in central ring (ring 1) in subjects with AMD compared to control group, whereas it did not show a significant change in ring 2 ($p = 0.024$, $p = 0.062$, respectively). N/Np and P/N indices did not show a significant change between control group and subjects with AMD (for N/Np ring 1, $p = 0.15$; for N/Np ring 2, $p = 0.062$; for P/N ring 1, $p = 0.89$; for P/N ring 2, $p = 0.88$). The indices in subgroups for ring 1 are shown in Table 3. Though N/Np index was found to be decreased in subjects with late AMD compared to control group and subjects with early/intermediate AMD, there was no statistically significant difference among groups. P/Pp index in ring 1 did not show any significant difference between control group and subjects with early/

**Table 2** Quadrant analysis in groups

| Parameter | Control ($n = 37$) | Early/int AMD ($n = 15$) | Late AMD ($n = 27$) | $p$ |
|---|---|---|---|---|
| N1 amplitude—SN | 16.8 ± 4.1[a] | 10.9 ± 6.3[b] | 12.1 ± 4.6[b] | <0.001 |
| N1 amplitude—ST | 19.5 ± 4.7[a] | 12.8 ± 6.8[b] | 13.8 ± 5.5[b] | <0.001 |
| N1 amplitude—IN | 20.2 ± 4.8[a] | 13.1 ± 6.3[b] | 14.6 ± 4.3[b] | <0.001 |
| N1 amplitude—IT | 24.1 ± 6.3[a] | 15.4 ± 6.8[b] | 16.3 ± 5.7[b] | <0.001 |
| N1 implicit time—SN | 26.1 ± 4.9 | 27.4 ± 2.6 | 27.4 ± 2.8 | 0.35 |
| N1 implicit time—ST | 26.0 ± 5.0 | 27.4 ± 4.9 | 27.3 ± 2.3 | 0.38 |
| N1 implicit time—IN | 24.8 ± 5.7 | 26.5 ± 2.3 | 26.7 ± 2.1 | 0.17 |
| N1 implicit time—IT | 25.1 ± 4.9 | 25.9 ± 3.5 | 26.4 ± 2.3 | 0.44 |
| P1 amplitude—SN | 30.4 ± 8.3[a] | 20.9 ± 10.9[b] | 22.2 ± 9.3[b] | <0.001 |
| P1 amplitude—ST | 35.1 ± 8.9[a] | 25.5 ± 11.9[b] | 25.4 ± 10.4[b] | <0.001 |
| P1 amplitude—IN | 38.0 ± 8.6[a] | 25.3 ± 12.9[b] | 27.5 ± 9.4[b] | <0.001 |
| P1 amplitude—IT | 43.3 ± 12.1[a] | 29.3 ± 14.4[b] | 31.3 ± 11.2[b] | <0.001 |
| P1 implicit time—SN | 44.1 ± 4.9[ns] | 47.4 ± 5.3[ns] | 46.5 ± 3.1[ns] | 0.025 |
| P1 implicit time—ST | 44.2 ± 5.0[a] | 48.1 ± 6.5[b] | 46.3 ± 2.8[a,b] | 0.023 |
| P1 implicit time—IN | 43.3 ± 4.7[ns] | 45.8 ± 3.7[ns] | 45.6 ± 2.8[ns] | 0.031 |
| P1 implicit time—IT | 43.5 ± 5.1 | 44.6 ± 5.2 | 45.4 ± 2.6 | 0.24 |
| N2 amplitude—SN | 24.6 ± 8.5[a] | 16.3 ± 9.4[b] | 16.8 ± 8.1[b] | 0.001 |
| N2 amplitude—ST | 26.8 ± 9.1[a] | 20.0 ± 9.4[b] | 20.4 ± 7.2[b] | 0.011 |
| N2 amplitude—IN | 30.1 ± 8.9[a] | 18.8 ± 11.3[b] | 20.4 ± 8.4[b] | <0.001 |
| N2 amplitude—IT | 32.0 ± 11.5[a] | 24.6 ± 12.7[a,b] | 22.0 ± 9.6[b] | 0.004 |
| N2 implicit time—SN | 60.8 ± 4.8[a] | 64.3 ± 5.1[a,b] | 65.8 ± 7.9[b] | 0.007 |
| N2 implicit time—ST | 61.5 ± 5.4[a] | 68.5 ± 10.4[b] | 64.2 ± 5.4[a,b] | 0.006 |
| N2 implicit time—IN | 60.9 ± 4.8 | 63.7 ± 6.8 | 63.0 ± 3.4 | 0.10 |
| N2 implicit time—IT | 61.4 ± 5.6[a] | 68.3 ± 10.9[b] | 65.5 ± 7.0[a,b] | 0.010 |

Data are expressed as mean ± SD; AMD, age-related macular degeneration; early/int, early/intermediate; SN, superior nasal; ST, superior temporal; IN, inferior nasal; IT, inferior temporal; ns, not significant; $p$, one-way ANOVA

Different superscript letters (a, b) represent a significant difference between groups according to Sheffe post hoc test. Values that are not significantly different have the same superscript letters

intermediate AMD. Though P/Pp index was found to be decreased in subjects with late AMD compared to both control group and subjects with early/intermediate AMD, the difference was only statistically significant between control group and subjects with late AMD.

## Discussion

AMD is an important cause of irreversible central vision loss in subjects aged over 55 years [9]. Linear thickness of Bruch's membrane increases with aging from 2 μm at birth to 4–6 μm at tenth decade [10]. In AMD eyes, RPE cells are under oxidative stress [11]. Lipofuscin accumulates within RPE cells with aging, which is greatest under the parafoveal retina, and the major source of lipofuscin is the undegradable products of photoreceptor outer segment metabolism [12]. Excessive accumulation of lipofuscin in RPE, which causes cell damage, is thought to play an important role in the pathogenesis of AMD [13]. The abnormal accumulation of lipofuscin between RPE and Bruch's membrane can induce a mechanical displacement of the outer segments of the photoreceptors and/or a defect of the nutrient exchange between photoreceptors and choriocapillaris [14, 15]. These changes can be related with impairment of macular photoreceptor functions. The evaluation of retinal functions may play an important role in understanding the mechanism of AMD and monitoring treatment modalities. Full-field ERG studies have shown a decrease in amplitudes and a delay in implicit times of ERG responses with age [16–18]. Full-field ERG results are normal until at least 20 % of the retina is affected and a legally blind person because of AMD may have a normal full-field ERG [19]. In these cases, multifocal ERG plays an important role. Multifocal

**Table 3** Indices in groups

| Parameter | Control ($n = 37$) | Early/int AMD ($n = 15$) | Late AMD ($n = 27$) | $p$ |
|---|---|---|---|---|
| N/Np ring 1 | 2.61 ± 0.9 | 2.60 ± 1.1 | 2.11 ± 0.9 | 0.11 |
| P/Pp ring 1 | 2.88 ± 0.9[a] | 2.70 ± 1.8[a,b] | 2.01 ± 1.1[b] | 0.016 |
| P/N ring 1 | 2.03 ± 0.3 | 2.45 ± 2.4 | 1.73 ± 0.5 | 0.14 |

Data are expressed as mean ± SD; N/Np, the ratio of amplitude between central N1 and peripheral N1; P/Pp, the ratio of amplitude between central P1 and peripheral P1; P/N, the ratio of amplitude between central P1 and central N1; ring 1, from 0° to 5° of eccentricity relative to fixation; AMD, age-related macular degeneration; early/int, early/intermediate; ns, not significant; $p$, one-way ANOVA

Different superscript letters (a, b) represent a significant difference between groups according to Sheffe post hoc test. Values that are not significantly different have the same superscript letters

ERG allows the identification and localization of defects localized in a limited area of visual field.

In this study, we evaluated responses from 61 retinal locations in subjects with AMD using mfERG. As high noise shows poor electrode contact, poor grounding or ambient sources, we did exclude recordings with a noise level higher than 5 μV. Mean noise was 2.3 ± 0.5 μV in control group and 2.4 ± 0.5 μV in subjects with AMD, which showed that the recordings were reliable in all subjects. None of our subjects had any media opacity. An important drawback of our study is that we used hexagons of equal dimensions during the test instead of scaled hexagons. In the test with hexagons of equal dimensions, the size of rings is 4.9°. For this reason, we could not evaluate central 5° as central 2° and central 2°–5° separately, which can be done when scaled hexagons are used.

In our study, concentric ring analysis of mfERG demonstrated a significant reduction in N1, P1 and N2 amplitudes in both early/intermediate AMD and late AMD subjects compared to control group, whereas there was no significant difference between subjects with early/intermediate AMD and late AMD. Implicit time of N1 was not affected in both early/intermediate AMD and late AMD subjects compared to control group. Implicit time of P1 was not changed in subjects with early/intermediate AMD compared to control group in central 5°–15°, whereas it was prolonged in subjects with late AMD compared to control group. Implicit time of N2 did not show a significant change in subjects with early/intermediate AMD compared to control group in central 10°, whereas it was prolonged in subjects with late AMD compared to control group. We could detect no difference in implicit times of N1, P1 and N2 between subjects with early/intermediate AMD and late AMD. It is known that in mfERG, the amplitudes are largest at the foveal area where cone photoreceptors and bipolar cells are densest [8]. The decrease in amplitude represents photoreceptor loss, whereas the delay in implicit time reflects disorders in the inner retina [20]. As a result, amplitudes of N1, P1 and N2 were found to be decreased in all rings in subjects with AMD (early/intermediate and late AMD), whereas implicit times were only affected in subjects with late AMD. This result made us think that though photoreceptors are affected at early stages of AMD, inner retina is being affected at later stages. Quadrant analysis showed that N1, P1 and N2 amplitude decreased significantly in all quadrants, whereas N1 implicit time was normal and P1 and N2 implicit times were prolonged in subjects with late AMD. Parisi et al [21] showed that mfERG amplitudes were decreased in central fovea and central 5° in subjects with AMD, whereas no significant change was recorded in 5°–20° areas. Similarly, Zol'nikova et al [22] reported that N1 and P1 amplitudes were decreased at the foveal and parafoveal area in subjects with focal RPE atrophy. Huang et al [23] compared mfERG recordings from 17 subjects with AMD (8 early/intermediate AMD, 9 late AMD) and 17 normal control subjects and reported decrease in amplitudes and a delay in implicit times. They concluded that these changes were mild in early AMD and more pronounced in late AMD. In our study, though implicit times showed an increase in subjects with early/intermediate AMD, there was no statistically significant difference compared to control group. Implicit times were only affected significantly in subjects with late AMD, whereas amplitudes were affected in both early/intermediate AMD and late AMD. So, inner retinal function is being affected at later stages in AMD. Garcia-Garcia et al [24] evaluated ten patients with drusen by mfERG and reported that implicit time of N1 and P1 was delayed in subjects with drusen compared to control group, whereas amplitudes were lower in the foveal area. Gerth et al [25] showed that there was a dysfunction in cone-driven pathway in subjects with large drusen as there was a delay in

implicit time and a reduction in amplitudes, where these changes were not correlated with morphologic findings and mfERG results were abnormal in areas with and without morphologic changes. Chen et al [26] showed that rod functions were especially affected at the parafoveal area (central 2.5°–5°) and scotopic responses were more affected than photopic responses.

As a result, mfERG is useful in evaluating retinal functions in subjects with AMD. Though dysfunction of photoreceptors is seen at early/intermediate AMD, at the late stages, inner retinal dysfunction occurs additionally to dysfunction of photoreceptors.

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**Conflict of interest** The authors have no other conflict of interest.

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