Comparison Of Multifocal Electroretinography Results With Two Different Electrodes In Patients Using Hydroxychloroquine: A Preliminary Study

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ABSTRACT

Purpose: To evaluate retinal electrophysiological responses with two different electrodes in patients who have been using HCQ at a dose of least 200 mg/day for more than five years and who are not known to have hydroxychloroquine (HCQ) retinal toxicity and to compare JET electrode data and H-K loop electrode data.

Method: 20 eyes of 20 participants, 10 patients and 10 controls to whom HCQ treatment will be started, who were admitted consecutively to the clinic between October 2020 and March 2021 and who met the inclusion criteria were evaluated prospectively.

Results: When the P1 wave amplitudes of both electrodes in the patient and control groups were compared, it was obtained lower in all waves in the patient group; Statistically significant difference was observed only in Ring 1 (p=0.029) in JET electrode, in ring 2 (p=0.019) and ring 3 (p=0.009) in H-K loop electrode.

Discussion: The reason for the variation of the affected ring with respect to the electrode may be associated with the recorded localization of the electrode. In this respect, internal consistency of the electrodes draws attention.

Keywords: hydroxychloroquine, electroretinography, electrodes

INTRODUCTION

Hydroxychloroquine (HCQ) (PLAQUENIL 200 mg film-coated tablet, Sanofi, Fawdon/UK) is widely used in the treatment of inflammatory diseases such as systemic lupus erythematosus and rheumatoid arthritis.[1] When used at a daily dose of 5.0 mg/kg or less, the annual risk of retinotoxicity is below 1% in the first ten years; however, after 20 years this risk approaches nearly 4%, and at doses exceeding 5.0 mg/kg per day, the risk increases by 2–3 fold.[2] For routine primary screening, the use of both visual field testing and optical coherence tomography (OCT) is recommended. Multifocal electroretinography (mfERG) can objectively document electroretinogram depression in early retinopathy.[1]

Multifocal electroretinogram is an electrophysiological test that enables simultaneous assessment of the function of multiple retinal regions.[3] During electroretinography (ERG) recording, electrodes in contact with the cornea, conjunctiva, or skin are used as active electrodes. Contact lens electrodes applied to the cornea provide the highest amplitude and the most stable recording; however, they are uncomfortable for the patient and may become displaced during the procedure. Conjunctival electrodes offer ease of application and comfort, but their amplitude values are lower. Reference amplitude ranges for standard ERG waves are electrode-specific.[4] The JET electrode is in the form of a contact lens, and its ring-shaped gold material enables recording of electrical activity from the cornea.[5]

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The H-K loop electrode is ring-shaped and is a conjunctival electrode made of silver coated with teflon.[6]

Our aim is to evaluate the retinal electrophysiological responses with two different electrodes in patients who have been using HCQ at a dose of at least 200 mg/day for more than five years and whose HCQ retinal toxicity status is unknown, and to compare the data obtained from the JET electrode with those from the H-K loop electrode.

MATERIALS AND METHODS

A total of 20 eyes were prospectively evaluated, including 10 patients who consecutively presented to our clinic between October 2020 and March 2021 and met the inclusion criteria, as well as 10 voluntary controls scheduled to initiate HCQ therapy. Only the right eyes of participants were included in the study. Ethical approval was obtained from the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine (Decision number: OMÜ KAEK 2020/378). The study adhered to the principles of the Declaration of Helsinki. Prior to the procedures, all participants were informed, and written informed consent was obtained.

Inclusion and exclusion criteria

All patients who had been regularly using at least 200 mg/day of HCQ for more than 5 years were included in the patient group. The control group consisted of volunteers within the same age range as the patient group, who were about to initiate HCQ therapy and had normal ophthalmological examinations.

Patients with any pathology detected on ophthalmological examination, a history of ocular surgery or trauma, central nervous system pathology, the use of non-HCQ medications that could cause retinal toxicity, or refractive errors greater than two diopters were excluded from the study.

Evaluation parameters

The following data were recorded for all participants: age, sex, systemic disease requiring HCQ treatment and disease duration, total duration of HCQ use, daily dose, cumulative dose, and body mass index. All patients underwent a detailed ophthalmological examination. Pupils were dilated with 1% tropicamide (1% TROPAMID, 5 ml drop, Bilim İlaç, Istanbul/Turkey). Macular OCT imaging (Spectralis HRA OCT, Heidelberg Engineering, Heidelberg, Germany) was then performed. Participants were kept in normal room illumination for 15 minutes, followed by mfERG recording with the H-K loop electrode (Vision Electrophysiology, France). Three to seven days after this examination, mfERG recording was repeated using the JET electrode (Vision Electrophysiology, France). All recordings were performed between 9:00 and 11:00 a.m. For mfERG, topical anesthesia with Proparacaine HCl 0.5% (ALCAINE 0.5% Sterile Ophthalmic Solution, 15 ml, Alcon, Puurs/Belgium) was applied, and only the right eye data of participants were analyzed. In all patient and control recordings, it was verified that noise levels were below 5 µV, the waveforms included a distinct peak indicating central fixation, and stable central fixation was continuously monitored using the built-in device camera.

Multifocal electroretinography

Multifocal ERG recordings were obtained using the Metrovision MonPack One (Vision Monitor Mon2014 D-4 ruedes planates, Perenchies, France) in accordance with ISCEV standards.[3] P1 wave amplitude and implicit times derived from the averaged signals of each hexagon were evaluated based on first-order kernel analysis. The test duration for each active electrode was approximately 5 minutes, with a total of 5004 stimuli delivered per mfERG recording.

Parameters compared in multifocal ERG recordings

- JET electrode data (P1 wave amplitude and implicit times) were compared between patient and control groups.
- H-K loop electrode data (P1 wave amplitude and implicit times) were compared between patient and control groups.
- The ring-to-ring 5 ratio of P1 wave amplitude was compared separately for both electrodes between patient and control groups.
- The ring-to-ring 5 ratio of P1 wave amplitude was compared separately for both electrodes between retinotoxic patients and controls.
- The proportioning of P1 wave amplitude values obtained from two different electrodes was done.

Statistical analysis

Statistical analysis was performed using SPSS Version 22 (IBM SPSS Statistics 22.0). Median, minimum, and maximum values of the variables were calculated as descriptive statistics. The Shapiro–Wilk test was used to assess the normality of numerical variables. For data not showing normal distribution, the Mann–Whitney U test was applied. A p-value <0.05 was considered statistically significant for all analyses.

Results

Demographic data

All participants were female, and the best corrected visual acuity was 1.0 (Snellen chart) in all cases. Age and refraction results between the groups are presented in Table 1.

Data on systemic diseases and HCQ dosage are presented in Table 2. In two patients with detected toxicity, HCQ treatment was immediately discontinued following consultation with the Rheumatology Department.

Comparison of results obtained with multifocal ERG

When P1 wave amplitudes of both electrodes were compared between the patient and control groups, lower values were observed across all waves in the patient group. A statistically significant difference was found only at Ring 1 (R1) with the JET electrode (p = 0.029), and at Ring 2 (R2) (p = 0.019) and Ring 3 (R3) (p = 0.009) with the H-K loop electrode (Table 3).

Table 1. *Age and refraction data in patient and control group*

		n	Median	Minimum	Maximum	p*
Age (years)	Patient	10	50,50	41	60	0,732
	Control	10	50,50	43	57	
Spherical (diopter)	Patient	10	+0,25	-0,75	+2,00	0,468
	Control	10	+0,37	-1,50	+2,00	
Cylindirical (diopter)	Patient	10	-0,62	0	-1,00	0,528
	Control	10	-0,50	0	-1,00	

*Mann-Whitney U test, p<0,05

Table 2. *Patients' demographic and drug use data and test results*

Patient	Age/ gender	Disease	Height (m)	Weight (kg)	BMI (kg/ m^2)	Total use/ diagnosis time (years)	Cumulative dose (g)	Weight-dose (mg/kg)	OCT	mfERG	HCQ use
1	43/F	Sjögren	1,58	67	26,84	8	1095	5,6	AN	AN	Discontinued
2	43/F	S(-)RA	1,50	46	20,44	11	1606	8,7	N	AN	Discontinued
3	58/F	RA	1,59	86	34,02	11	1606	4,65	N	N	Continues
4	60/F	RA	1,50	77	34,22	12	1820	5,4	N	N	Continues
5	45/F	SLE	1,62	78	29,72	9	1200	4,68	N	N	Continues
6	57/F	Sjögren	1,55	80	33,30	6	876	5	N	N	Continues
7	56/F	S(-)RA	1,58	90	36,05	8	1168	4,44	N	N	Continues
8	41/F	Sjögren	1,68	49	17,36	7	1022	8,16	N	N	Continues
9	54/F	SLE	1,50	85	37,78	9	1314	4,71	N	N	Continues
10	47/F	CTD	1,60	96	37,50	11	1520	3,94	N	N	Continues
Median	50,5	-	1,58	79	33,66	9	1257	4,85	-	-	-

BMI:Body mass index, OCT:Optic coherence tomography, mfERG: Multifocal electroretinography, HCQ: Hydroxychloroquine, F:Female, S(-)RA: Seronegative rheumatoid arthritis, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus , CTD: Collagen tissue disease, AN:Abnormal, N: Normal

Table 3. *Comparison of JET electrode and H-K loop electrode P1 wave amplitudes between patient and control group*

JET electrode					
P1 vawe Rings	Patient		Control		p*
	Median	Min-Max	Median	Min-Max	
R1	1307	591-2201	1993	1605-3054	0,029
R2	1231	533-1598	1300	1140-1793	0,218
R3	1159	692-1643	1178	1026-1644	0,393
R4	1196	1028-1676	1217	987-1678	0,631
R5	1311	1076-1857	1323	1038-1823	0,853

*Mann-Whitney U, units nV, p<0,05

H-K loop electrode					
P1 wave Rings	Patient		Control		p*
	Median	Min-Max	Median	Min-Max	
R1	893	304-1265	1044	597-1918	0,190
R2	513	365-604	623	477-870	0,019
R3	429	250-537	541	415-911	0,009
R4	459	269-586	527	345-807	0,353
R5	511	258-592	521	411-785	0,456

*Mann-Whitney U, units nV, p<0,05

When implicit times of the P1 wave were compared between electrodes, delayed responses were observed across all waves in the patient group, but no statistically significant differences were detected.

When the ratios of P1 wave amplitudes obtained from each ring to the amplitude of Ring 5 (R5) were compared between the patient and control groups, all ratios were lower in the patient group for both electrodes (Table 4). A statistically significant difference was found only for the R1/R5 amplitude ratio obtained from the JET electrode (p = 0.041).

When the ratios of P1 wave amplitudes obtained from each ring to the amplitude of R5 were compared between patients with retinotoxicity and the control group, all ratios were lower in the retinotoxic patient group for both electrodes (Table 5). Statistically significant differences were observed for the R2/R5 and R3/R5 ratios obtained from the JET electrode, and for the R2/R5 ratio obtained from the H-K loop electrode (all p = 0.032).

The ratios of amplitude values obtained from the H-K loop electrode to those obtained from the JET electrode are presented in Table 6.

Table 4. *The ratio of P1 wave amplitude rings to ring 5 in JET and H-K loop electrode between patient and control group*

	JET electrode					**H-K loop electrode**				
Ring ratio	Patient		Control		p*	Patient		Control		p*
	Median	Min-Maks	Median	Min-Maks		Median	Min-Maks	Median	Min-Maks	
R1/R5	1,11	0,47-1,82	1,62	1,12-2,74	0,041	1,80	0,51-4,57	1,86	1,40-2,96	0,762
R2/R5	0,86	0,45-1,27	0,99	0,81-1,40	0,082	0,98	0,74-1,74	1,14	0,94-1,28	0,257
R3/R5	0,87	0,63-0,98	0,92	0,72-1,11	0,199	0,87	0,59-1,47	1,01	0,80-1,26	0,070
R4/R5	0,89	0,81-1,00	0,91	0,83-1,03	0,218	0,95	0,74-1,22	0,96	0,71-1,04	1,000

*Mann-Whitney U, p<0,05

Table 5. *The ratio of P1 wave amplitude rings to ring 5 in JET and H-K loop electrode between retinotoxic patient and control group*

	JET electrode					H-K loop electrode				
Ring ratio	Retinotoxic Patient		Control		p*	Retinotoxic Patient		Control		p*
	Median	Min-Max	Median	Min-Max		Median	Min-Max	Median	Min-Max	
R1/R5	0,83	0,47-1,21	1,62	1,12-2,74	0,133	2,03	1,42-2,65	1,86	1,40-2,96	1,000
R2/R5	0,47	0,45-0,50	0,99	0,81-1,40	0,032	0,84	0,76-0,93	1,14	0,94-1,28	0,032
R3/R5	0,63	0,63-0,64	0,92	0,72-1,11	0,032	0,72	0,59-0,86	1,01	0,80-1,26	0,086
R4/R5	0,90	0,81-1,00	0,91	0,83-1,03	0,830	0,84	0,74-0,95	0,96	0,71-1,04	0,390

*Mann-Whitney U, p<0,05

Table 6. *The ratio of amplitude values obtained from H-K loop to amplitude values obtained from JET electrode*

Rings	H-K loop/JET for P1 wave			
	Patient		Control	
	Median	Min-Max	Median	Min-Max
R1	0,49	0,36-1,31	0,52	0,29-0,84
R2	0,45	0,36-0,90	0,44	0,28-0,57
R3	0,44	0,39-0,60	0,36	0,21-0,51
R4	0,39	0,28-0,57	0,41	0,22-0,59
R5	0,41	0,29-0,54	0,40	0,21-0,56

DISCUSSION

Although the prevalence of hydroxychloroquine (HCQ) retinopathy has been reported in the literature as low as 0.1–0.5%, it may cause irreversible vision loss; therefore, patients receiving this medication should be followed up regularly. The only treatment for HCQ retinotoxicity is discontinuation of the drug.[1,7]

In numerous studies, mfERG has been considered the gold standard test due to its objectivity and ability to demonstrate retinal responses with high sensitivity.[8-11] In this regard, mfERG holds particular importance among the screening tests for HCQ retinotoxicity.

The type of electrode used in mfERG testing is critical with respect to many parameters, such as recording quality, ease of application, patient comfort, and cost. Some electrodes provide high-quality recordings but are less comfortable for patients or may easily dislodge from the eye, while others are easier to use but yield waves of lower amplitude. Parameters such as amplitude and implicit time differ among electrodes, each having unique characteristics. The most accurate approach is for laboratories to establish their own normative values with their own devices and electrodes. Since electrophysiological data are not normally distributed, laboratories should report median values rather than mean values, and define normality ranges accordingly.[3,12,13] In our study, we also used median values and non-parametric tests for statistical evaluation of electrode-derived results, and we established our normative data from our own control group.

Hawlina et al.[6] reported that pattern ERG (pERG) wave amplitudes recorded with the H-K loop electrode were comparable to those obtained with gold foil electrodes, approximately two-thirds of those recorded with corneal electrodes, and more than three times greater than those obtained with skin electrodes. Nowitzki et al.[14] compared DTL and skin electrodes, noting that while the waveform patterns were similar, amplitudes were lower with skin electrodes, likely due to impedance from the skin and eyelid muscles. They suggested that skin electrodes could be an alternative in children or disabled patients where corneal electrodes are not feasible. Mann et al.[15] defined an ideal electrode as one that does not distort the signal or introduce additional noise. Gold corneal electrodes exhibit higher impedance compared to silver electrodes; when potential drift was examined over a 60-minute recording period, gold electrodes showed the most unstable drift, while silver electrodes stabilized more quickly. Potential drift was found to be less with skin electrodes than with ocular electrodes, and stabilization occurred more rapidly.[15]

The large number of parameters specific to mfERG may be considered a limitation of our study, as it complicates the evaluation of results. It was not possible to derive a single main comparison or establish a fixed amplitude ratio between these electrodes, as has been suggested in pERG. However, in general, amplitudes obtained with the H-K loop electrode corresponded to approximately 40–50% of those obtained with the JET electrode (Table 6).

García et al.[16] compared mfERG recordings obtained with three different electrodes (corneal-DTL, fornix-DTL, and JET) in terms of wave amplitude, number of artifacts, frequency of electrode displacement during the test, and discomfort level. They found that the JET electrode provided the highest amplitudes but was associated with greater discomfort and more frequent dislodgement. Esakowitz et al.[17] compared six different electrodes in four healthy volunteers regarding ERG recording quality, ease of placement, and patient comfort, concluding that none of the electrodes tested was entirely ideal. Parks et al.[12] aimed to establish normal values for mfERG using the H-K loop electrode and reported that it was as sensitive as the gold foil electrode and less uncomfortable than contact lens electrodes.

The aim of our study was to identify the most suitable electrode for mfERG testing, balancing sufficient comfort and recording quality. We compared data obtained from the more comfortable H-K loop electrode, which yields lower amplitudes, with those from the JET electrode. Despite many studies comparing ERG electrode types, our literature review revealed no previous study comparing JET and H-K loop electrodes in patients receiving HCQ and in healthy controls.

When P1 wave amplitudes recorded with the same electrode were compared between patient and control groups, statistically significant differences were found in R1 with the JET electrode and in R3 with the H-K loop electrode. The shift in the affected ring depending on the electrode may be related to electrode recording localization. The JET electrode has consistently been reported as providing higher amplitudes, likely due to the cornea being a better conductor than the conjunctiva or sclera, as well as the use of low-resistance material such as gold in its design.[17] Furthermore, electrode position on the eye is a critical factor influencing amplitude. Otto and Bach[18], using DTL electrodes in pattern ERG, reported amplitudes 20% higher when the electrode was placed across the cornea rather than in the inferior fornix. The cornea is a better conductor than the conjunctiva, and amplitudes increase as the electrode is positioned closer to the corneal center.[19] Amplitude differences between electrodes have also been shown to relate to the distance between the eye and the recording site.[20] In mfERG, variation in affected rings may therefore be explained by the conductivity of the cornea versus conjunctiva, or possibly by additional interactions involving electrode distance and position relative to the fovea.

Multifocal ERG ring ratios provide sensitive and objective data that can confirm clinical suspicion of reduced visual function and serve as early predictors of impending retinotoxicity in long-term HCQ users. Expressing responses as ratios has several advantages: ratios are minimally influenced by age, stimulus brightness, reference electrode position, or anterior segment differences, and they correlate more reliably with reduced retinal function.[21] Ratios between rings remain relatively stable in healthy retinas. Nevertheless, each laboratory should establish its own normative values. Ring 1 typically exhibits the greatest variability among subjects, being based on a single response and most susceptible to noise; therefore, it is not recommended to use R1 as a denominator when employing ring ratios.[3]

In our study, when comparing ratios of amplitudes obtained from each ring to that of R5, lower ratios were found in the patient group across both electrodes. A statistically significant difference was detected only in the R1/R5 ratio with the JET electrode ($p = 0.041$, Table 4). The R3/R5 ratio with the H-K loop electrode did not reach statistical significance ($p = 0.07$, Table 4) but approached significance, again highlighting electrode-specific consistency.

When these ratios were compared between retinotoxic patients and controls, lower ratios were observed in the patient group across both electrodes (Table 5). Statistically significant differences were found in the R2/R5 and R3/R5 ratios with the JET electrode and in the R2/R5 ratio with the H-K loop electrode (all p = 0.032). Tsang et al.[22] reported that the R2/R5 ring ratio is a strong indicator of retinotoxicity and demonstrated an inverse relationship between cumulative dose quartiles and the R2/R5 ratio. Our results, consistent across both electrodes, support these findings. The high cumulative doses in our patient group may account for this outcome. Furthermore, obtaining consistent results across both electrodes suggests that ring amplitude ratios may provide more objective assessments than direct amplitude comparisons alone.

Based on our findings, the JET electrode clearly demonstrates retinal pathology due to its higher amplitudes; however, the H-K loop electrode also appears valuable in detecting retinal pathology once normative data are established.

Although the number of patients and volunteers in our study may be considered small, the sample size exceeded that recommended by pre-study power analysis. The number of patients with retinotoxicity was limited; however, our study was designed to evaluate HCQ users regardless of known toxicity status. Our primary aim was to compare data obtained from two different electrodes against our control group's normative values.

The fact that our study included only female participants could be regarded as a limitation, although this occurred by chance. With increasing age, ERG amplitudes have been reported to decrease and implicit times to lengthen in both genders; women were found to have higher amplitudes and shorter implicit times compared to men.[23,24] Therefore, studying a single gender within a similar age range may actually be advantageous.

CONCLUSION

Multifocal ERG is highly valuable for the early detection of HCQ retinotoxicity. The H-K loop electrode, due to its easier application, may replace other electrodes and be preferable in more patients owing to increased comfort. With larger sample sizes, reliable results in detecting HCQ toxicity can be achieved, and the H-K loop electrode may be favored over others. This emphasizes the importance of each electrophysiology laboratory establishing its own normative values, ideally through the creation of large databases with as many participants as possible.

In Patient 1, in whom retinotoxicity was detected, abnormal values in N1 and P1 amplitudes were observed in R1 and R2 with the JET electrode, while abnormal values were seen in R2 and R3 with the H-K loop electrode. According to our limited normative data, HCQ retinotoxicity could be diagnosed in this patient with either electrode (Figures 1 and 2).

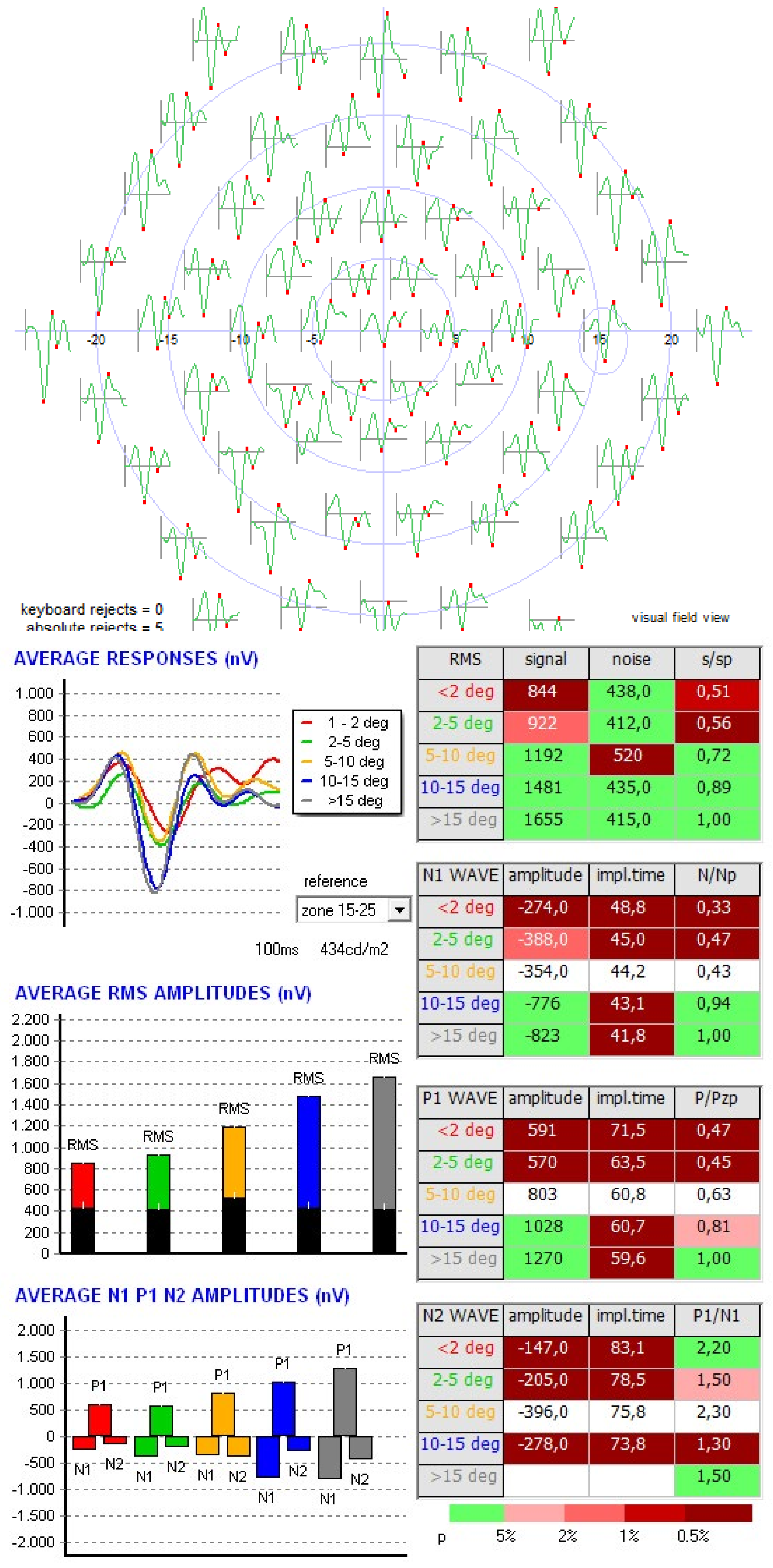

Figure-1: *Multifocal ERG recording with JET electrode of Patient 1 who was diagnosed with retinotoxicity*

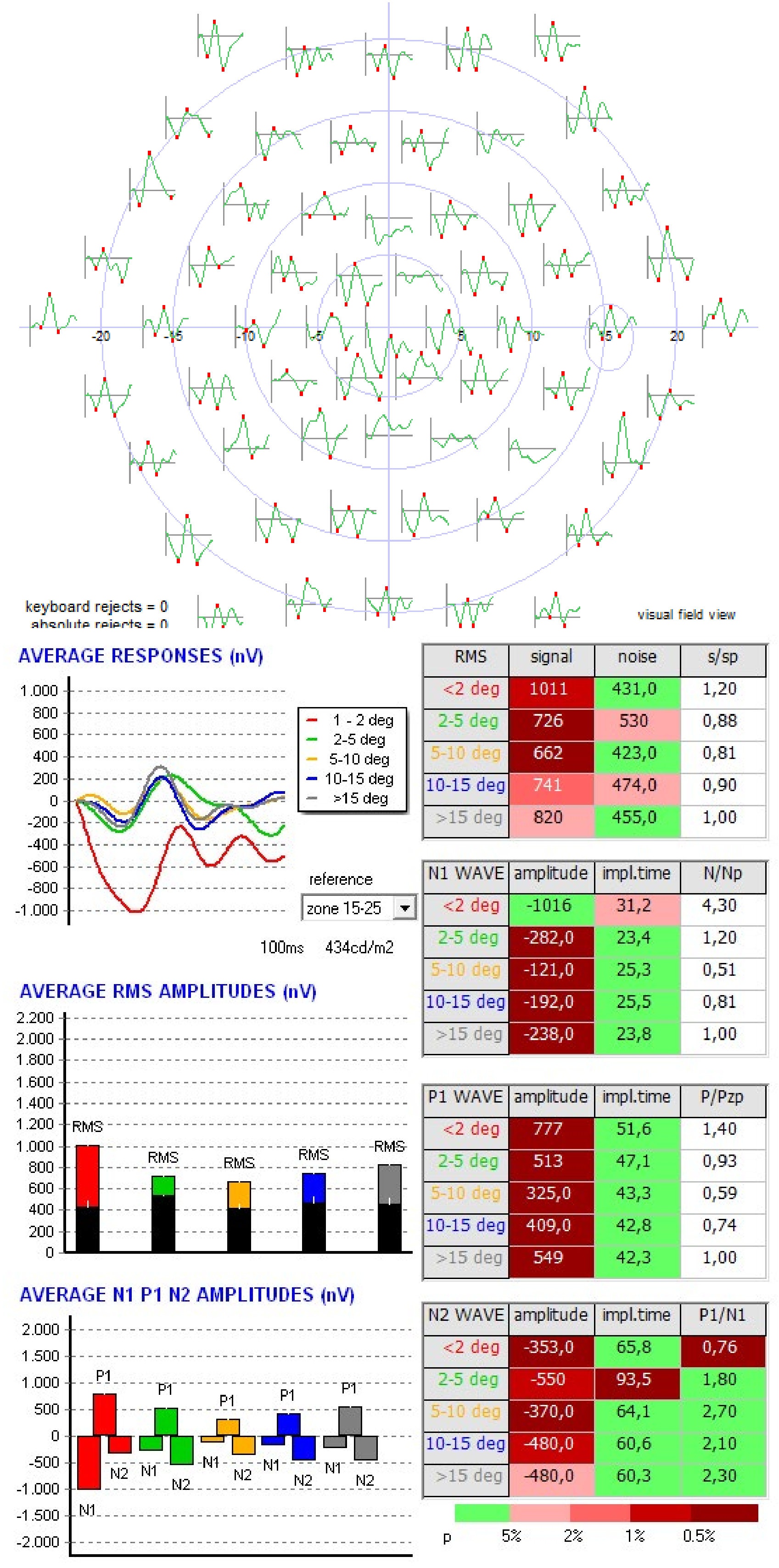

Figure-2: *Multifocal ERG recording with H-K loop electrode of Patient 1 with retinotoxicity*

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