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Article

Comparison of Fundus-Guided Microperimetry and Multifocal Electroretinography for Evaluating Hydroxychloroquine Maculopathy

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Purpose: To compare retinal function by using fundus-guided microperimetry (MP) and multifocal electroretinography (mfERG) for detecting hydroxychloroquine (HCQ) maculopathy.

Methods: Forty-six eyes of 25 patients referred to our clinical practice for HCQ maculopathy assessment and 3 groups of normal control subjects were evaluated by mfERG and MP. Macular structure was assessed using spectral-domain optical coherence tomography (SD-OCT). Ring ratios from the three innermost mERG rings were compared with average sensitivity of each MP ring at approximately equivalent distances from the fovea. HCQ toxicity was defined as an mfERG ring ratio or mean MP ring sensitivity >2 standard deviations below the normal mean. The sensitivity and specificity of MP to detect HCQ toxicity relative to mfERG were evaluated.

Results: MP rings MR2 and MR3 were positively correlated with corresponding mfERG ring ratios ($r = 0.52$, $P = 0.002$ and $r = 0.56$, $P < 0.001$ respectively). Ring 2 and ring 3 measures of MP and mfERG were significantly worse in HCQ eyes than controls ($P < 0.001$). The sensitivity of MP to detect toxicity for MR1 through MR3 ranged from 33% to 88%, whereas specificity ranged from 72% to 85%. Through rings 1 to 3, the frequency of abnormal function ranged from 20% to 48% for MP, 11% to 35% for mfERG, and 41% to 45% for SD-OCT.

Conclusions: The frequency of detection of HCQ toxicity with MP was greater than with mfERG. MP showed an overall good sensitivity and moderate specificity in detecting HCQ-induced functional deficits.

Translational Relevance: Results from this study may allow clinicians to improve screening accuracy for HCQ toxicity by using the alternative modality of MP.

Introduction

Hydroxychloroquine (HCQ; brand name Plaquenil) is widely used for the treatment of autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).[1] However, HCQ use may lead to the development of irreversible retinopathy. Early detection of toxicity and subsequent discontinuation of HCQ usage is crucial for limiting further progression and risk of central vision loss.[2] Screening tests currently recommended by the American Academy of Ophthalmology (AAO) include 10-2 static automatic perimetry (SAP) and spectral-domain optical coherence tomography (SD-OCT) as primary tests and multifocal electroretinography (mfERG) and fundus autofluorescence as recommended additional tests when available.[3] Recent literature has focused on determining which testing modalities and analysis methods produce data

that most appropriately reflect whether HCQ toxicity is occurring.[4–10] There is currently no conclusive screening test or standardized protocol for defining early signs of HCQ toxicity.

The mfERG is an objective test that provides a direct measure of cone-pathway-mediated retinal function, has been reported to be highly sensitive,[7,11] and is considered by some to be the gold standard test[8,12] for detecting cases of HCQ toxicity earlier than other testing modalities. HCQ maculopathy manifests as a ring of depressed responses in the perifoveal regions of the macula.[13,14] Average mfERG ring responses[8,13,14] and/or ratios between rings[4,7,8,15–17] have been used for the quantitative assessment of abnormalities in the mfERG as indicators of HCQ maculopathy. However, mfERG testing requires individuals with technical expertise to both perform the test and properly interpret the results, and its availability is generally limited to larger clinical centers. Fundus-guided microperimetry (MP) is another functional visual field test that measures retinal sensitivity and has the potential to detect changes consistent with HCQ retinal toxicity.

MP offers some technical advantages relative to mfERG; it requires less training, and the testing procedure is less invasive. In addition, it is recorded with simultaneous fundus tracking and allows for accurate quantification of visual sensitivity at specific points on the retina. Several groups have evaluated the ability of MP testing to detect HCQ-induced retinal toxicity.[5,6,9,18,19]. However, few studies have examined the sensitivity and specificity of MP for detecting HCQ retinopathy relative to mfERG (Khan NW, et al. *IOVS*. 2017;58:ARVO E-Abstract 5881).[12] Quantitative comparisons of mfERG and MP for detecting HCQ-induced toxicity are generally lacking, in terms of relative sensitivity and specificity and correlations between measures from these modalities. As such, the utility of MP for detecting HCQ toxicity is still uncertain

The purpose of this study was to compare and correlate retinal function measured using MP relative to that measured by mfERG as a reference test to evaluate the advantages and disadvantages of each modality for determining the presence of HCQ toxicity. Measures of retinal function from MP and mfERG were compared with quantitative SD-OCT retinal thickness measurements made at the same eccentricities from the fovea as the two inner mfERG and MP rings.Results from this study may significantly impact the approach clinicians currently use to evaluate early signs of HCQ toxicity.

Methods

The study protocol and the informed consent process were reviewed and prospectively approved by the Institutional Review Board of the University of Michigan Medical School. The study protocol complied with the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after explanation of the study and the possible risks.

Participants

Patients using HCQ (denoted from here on as HCQ participants) were recruited from the Retina Clinic of the Kellogg Eye Center, University of Michigan. Demographic data (Supplementary Table S1), medical history, height, body weight, diagnostic indication for HCQ use, and detailed history of HCQ dosing regimen were collected from the patient history, intake form, and secondarily from medical records. Patients with incomplete information on HCQ dosing, height or weight, and termination of HCQ treatment more than 6 months prior to exams were excluded. Enrollment criteria for normal controls were a Snellen visual acuity of 20/20 or better in each eye, a dilated eye exam within the past 12 months of participation in the study, and no history of HCQ use. All participants with diabetic retinopathy, age-related macular degeneration, significant cataracts, or refractive error exceeding ±6.0 diopters were excluded. In a subset of HCQ participants ($n = 17$), SD-OCT scans were obtained following mfERG and MP testing. Analysis of SD-OCT scans was conducted as an ancillary assessment to correlate retinal structure with function.

Multifocal Electroretinography

mfERG was performed following the recommendations of the International Society for Clinical Electrophysiology of Vision.[20] After pupil dilation (1% tropicamide and 2.5% phenylephrine) and topical anesthesia, mfERGs were recorded monocularly using Burian Allen electrodes (Hansen Ophthalmic Development Lab, Coralville, IA) on the VERIS 6.4 Science system (ElectroDiagnostic Imaging Inc., Milpitas, CA). The test stimulus consisted of 103 hexagons delivered at a rate of 75 Hz from a fixation monitoring system (FMS III) using a pseudorandom m-sequence on a background luminance of 200 cd.m^{-2}. The fixation target was a 3° diameter circle that bordered the central hexagon to avoid coverage of the stimulus. The average response density

amplitudes (nV/deg^2) from each of 6 inner concentric rings (91 stimulus areas) of the stimulus pattern were obtained.

Microperimetry

MP was performed after pupil dilation on the SD-OCT/confocal scanning laser ophthalmoscope Optos Microperimeter (formerly OPKO Inc., Dunfermline, Scotland) that automatically tracks fundus localization according to retinal vessel alignment to ensure proper placement of stimuli. Parameters included a Goldmann III size stimulus (area of 4 mm^2, diameter of 0.4 degrees), 200-ms duration of stimulus presentation, a 4-2 test strategy, and attenuated scale from 0 to 20 db. The Polar 3 stimulus grid, which assesses the central 12 degrees of the macular region, was used, and the sensitivity in decibels (dB) at each of the 28 points in the grid was obtained.

mfERG and MP Analysis

For full ring analysis, mfERG ring ratios were calculated as the average response density of the three innermost concentric rings R1, R2, or R3 divided by the mean of the outer ring R6 (referred to as R1/R6, R2/R6, and R3/R6, respectively). No spatial filtering was applied in order to maintain the integrity of localized responses. Mean MP retinal sensitivity (dB) was calculated for each MP ring MR1, MR2, and MR3. The areas covered by each mfERG ring were R1, 0° to 1°; R2, 1° to 4°; and R3, 4° to 8°; and the corresponding areas covered by the MP rings were MR1, 1°; MR2, 3°; and MR3, 5° (Fig. 1A). The three mfERG ring ratios and MP rings at approximately equivalent distances from the fovea were compared. Because full ring averages can mask partial losses, we also evaluated MP and mfERG responses from partial rings in the superior and inferior hemifields. Data points in R2 and R3 were divided into superior and inferior partial rings with stimuli along the horizontal midline excluded, forming four groups, namely, superior R2, superior R3, inferior R2, and inferior R3, each containing all points in either the superior or inferior subfields of a particular ring, and were referenced to corresponding points in R6 (Fig. 1B). Using the mfERG as the reference test, we considered the mfERG to be indicative of HCQ related maculopathy if (1) R1/R6, R2/R6, and R3/R6 or (2) R2/R6 and R3/R6 were more than 2 standard deviations (SDs) away from the normal mean. The MP was considered to have HCQ maculopathy if (1) MR1, MR2, and MR3 or (2) MR2 and MR3 were 2 SDs below the normal mean.

SD-OCT Imaging and Analysis

SD-OCT images in both eyes of a subset of HCQ participants were acquired using a Spectralis HRA-OCT device (Heidelberg Engineering Inc., Heidelberg, Germany). Transfoveal scans were obtained in the macular region, and the images were analyzed quantitatively by using the Heidelberg Eye Explorer software. The foveal center was identified as the reference for making measurements at 3° (900 μm) and at 6° (1800 μm) from the fovea, corresponding to R2 and R3 of mfERG and MP, respectively. Vertical thickness measures of the outer nuclear layer (ONL) and the Henle fiber layer (HFL) were made orthogonal to the retina through the foveal scan along the horizontal in the temporal and nasal retina at 3° and at 6°. Representative images showing the measurement scheme are shown in Figure 1C for a normal control and an HCQ participant. Thickness from the border of the outer plexiform layer to the border of the external limiting membrane (ELM) was denoted as OPELM. The HFL thickness (OPHF) was measured from the outer plexiform layer to the outer border of the HFL. The ONL thickness was measured from the border of the OPHF complex to the outer border of the ELM. Scans with oblique beam of entry (indicated by tilted scan) were excluded from analysis to avoid artifacts caused by HFL prominence, which would result in a false sense of ONL thinning. Foveal thickness was measured from the internal limiting membrane to the border of ELM. Quantitative assessment was performed independently by two masked readers (T.R.P. and M.F.A.).

Statistical Methods

Demographics of HCQ cases and controls were summarized using means, SDs, frequencies, and percentages. Differences between cases and controls were assessed with *t*-tests and Fisher exact tests. Linear mixed regression models (LMMs) were used to test for differences in mfERG, MP, and SD-OCT measures between HCQ participants and controls while accounting for the correlation between eyes of a subject.

An indicator of abnormal function was created by comparing measures on each HCQ eye to distributional characteristics of the same measure in a normal control sample. HCQ toxicity was defined as a measure >2 SDs from the normal mean. Imbalance

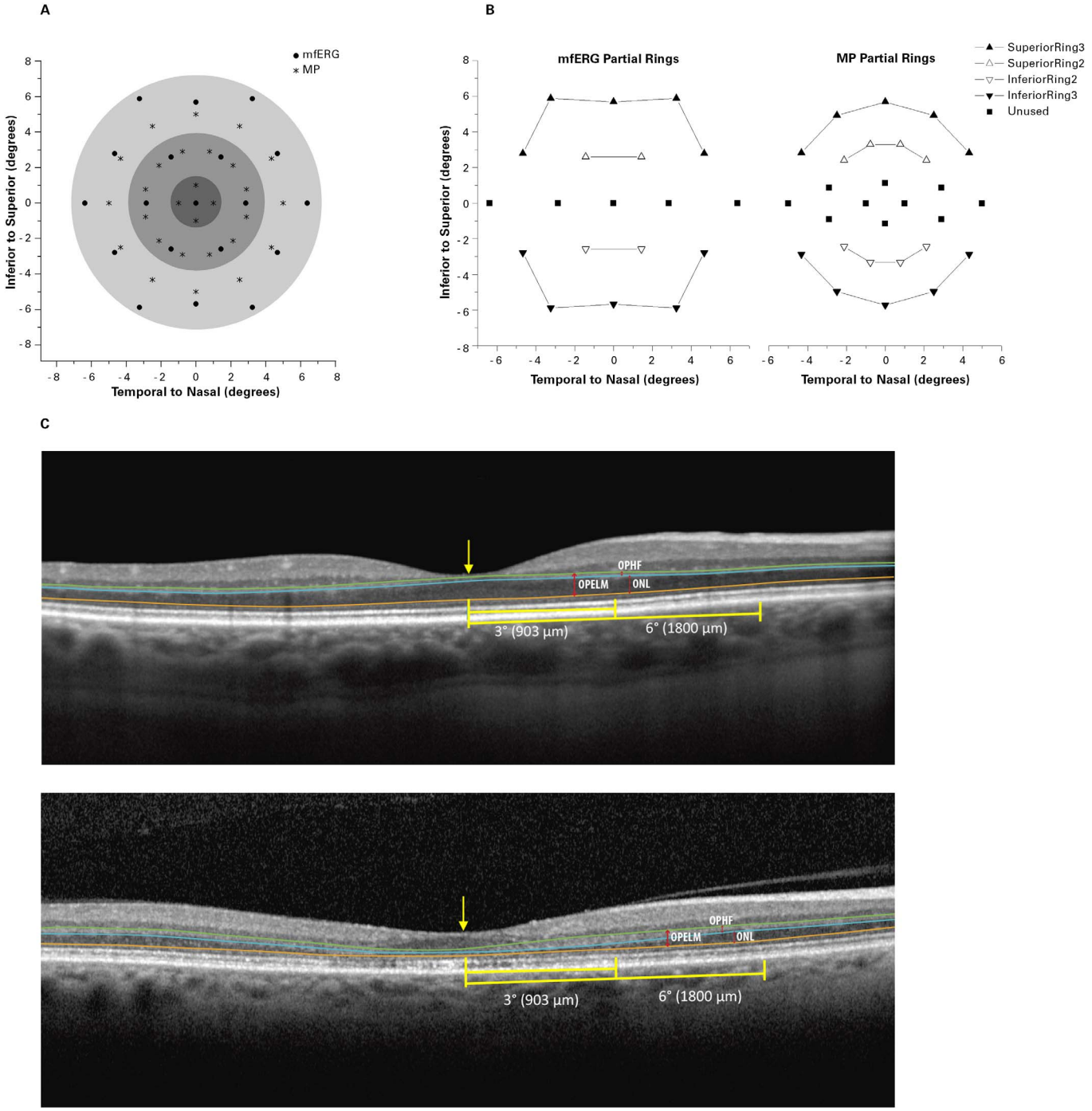

Figure 1. Retinal locations of multifocal ERG (mfERG) and microperimetry (MP) stimulus points. (A) Overlay of mfERG and MP stimulus points, arranged in a pattern of three concentric rings showing the locations of the innermost ring 1, ring 2, and ring 3 for each test. (B) A subset of points within rings 2 and 3 were used to determine responses from the superior or inferior hemifields. (C) SD-OCT images showing vertical thickness measurements of the Henle fiber layer (OPHF) and ONL were made in in the temporal and nasal retina at 3° and 6° from the fovea. OPELM: thickness from the border of the outer plexiform layer (OPL) to the border of the external limiting membrane (ELM). OPHF: thickness of Henle fiber layer (HFL) measured from the OPL to the outer border of HFL. ONL: thickness of outer nuclear layer was measured from the border of the OPHF complex to the outer border of ELM. *Top panel*: Normal control; *Lower panel*: Patient undergoing HCQ therapy.

Table 1. Patient Characteristics

Patient Characteristic	Mean ± SD	Range
Age (years)	56.6 ± 10.9	33.3–75.1
Height (m)	1.62 ± 0.09	1.44–1.93
Weight (kg)	76.5 ± 17.2	51.0–123.4
Daily dose (mg/kg/day)	5.3 ± 1.6	1.6–7.8
Cumulative HCQ dose (g)	1436.9 ± 882.7	219.2–2922
Length of treatment (years)	11.2 ± 6.4	1.5–25.0

in the abnormal classification between mfERG and MP measures, for corresponding rings, within HCQ eyes was tested with McNemar's tests. Impairment scores (Z-scores) were calculated for mfERG and MP rings from HCQ participants as the number of SDs away from the normal mean and compared for differences from normal participants with LMMs. Linear association between retinal measures by mfERG and MP, within the HCQ sample, was investigated with Pearson's correlations.

Sensitivity and specificity of MP measures to determine abnormal retinal function were calculated relative to abnormal function determined by mfERG in corresponding locations. Ninety-five percent Wilson confidence intervals (CI) are reported. Analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC) and R, version 3.2.1 (R Core Team, Vienna, Austria).

Results

Subject Populations

A total of 46 eyes of 25 HCQ participants were included in the study (Table 1). All patients were undergoing HCQ therapy for either RA or SLE (64%), for both (20%), or for Sjogren's or sarcoidosis (16%). Snellen visual acuity ranged from 20/20 to 20/50 (median = 20/25). Of the 25 HCQ participants, SD-OCTs were studied from 29 eyes of 17 patients. Three separate samples of control participants of similar age as HCQ participants underwent mfERG, MP, or SD-OCT testing (Supplementary Table S1). HCQ participants were predominantly female, with 88% female and 12% male (Supplementary Table S1). Among the normal participants, mfERG group 1 controls were 78% female and MP group 2 controls were 78.6% female. However, in the SD-OCT group 3 controls, the majority were males (62%) (Supplementary Table S1). Age was not significantly different between HCQ participants and the three control samples (all $P > 0.05$). Representative mfERG, MP, and SD-OCT results from a normal control and an HCQ participant are shown in Figure 2, illustrating the HCQ-associated parafoveal loss on mfERG and MP.

Reduced Function and Structural Changes in HCQ Participants

For the HCQ eyes, MP sensitivity (dB) was significantly correlated with the corresponding

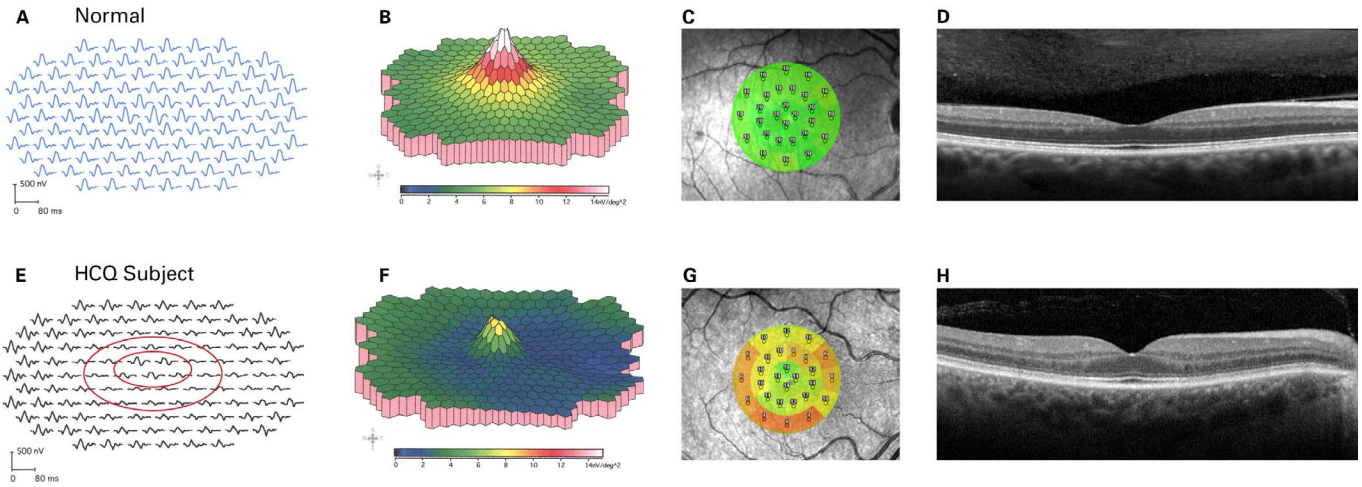

Figure 2. Comparisons of normal versus abnormal outcomes for mfERG, MP, and SD-OCT measurements. Examples of (A) mfERG trace array, (B) mfERG response density plot, (C) microperimetry sensitivity map, and (D) SD-OCT scan for a normal control. In comparison, a patient with HCQ retinopathy showing (E, F) depressed mfERG responses in the parafoveal region, (G) decreased MP sensitivity (dB) in the parafoveal region, and (H) thickening of the Henle fiber layer and thinning of the outer nuclear layer by SD-OCT.

Table 2. Descriptive Statistics on mfERG, MP, and SD-OCT Measures, Stratified by Patient Sample

	HCQ Eyes			Normal Eyes			
Measure	Mean (SD)	Normal Frequency (%)	Abnormal Frequency (%)	Mean (SD)	Normal Frequency (%)	Abnormal Frequency (%)	*P* Value[a]
mfERG[b]							
R1/R6	3.08 (0.93)	34 (73.9)	12 (26.1)	3.60 (0.60)	59 (98.3)	1 (1.7)	0.007
R2/R6	2.01 (0.58)	32 (69.6)	14 (30.4)	2.41 (0.38)	59 (98.3)	1 (1.7)	<0.001
R3/R6	1.35 (0.38)	30 (65.2)	16 (34.8)	1.65 (0.23)	60 (100.0)	0 (0.0)	<0.001
Superior R2/R6	2.22 (0.65)	41 (89.1)	5 (10.9)	2.43 (0.52)	60 (100.0)	0 (0.0)	0.13
Superior R3/R6	1.39 (0.42)	33 (71.7)	13 (28.3)	1.64 (0.25)	58 (96.7)	2 (3.3)	0.001
Inferior R2/R6	1.76 (0.60)	39 (84.8)	7 (15.2)	2.36 (0.66)	60 (100.0)	0 (0.0)	<0.001
Inferior R3/R6	1.32 (0.37)	41 (89.1)	5 (10.9)	1.70 (0.42)	60 (100.0)	0 (0.0)	<0.001
MP[c]							
MR1	15.10 (2.74)	37 (80.4)	9 (19.6)	16.43 (2.17)	52 (96.3)	2 (3.7)	0.02
MR2	14.37 (2.60)	28 (60.9)	18 (39.1)	16.68 (1.30)	51 (94.4)	3 (5.6)	<0.001
MR3	13.30 (2.85)	24 (52.2)	22 (47.8)	16.29 (1.33)	51 (94.4)	3 (5.6)	<0.001
Superior MR2	14.28 (2.70)	26 (56.5)	20 (43.5)	16.58 (1.35)	53 (98.2)	1 (1.8)	<0.001
Superior MR3	13.51 (2.66)	25 (54.4)	21 (45.6)	16.17 (1.39)	51 (94.4)	3 (5.6)	<0.001
Inferior MR2	14.32 (2.73)	32 (69.6)	14 (30.4)	16.57 (1.63)	51 (94.4)	3 (5.6)	<0.001
Inferior MR3	13.03 (3.13)	24 (52.2)	22 (47.8)	16.26 (1.51)	50 (92.6)	4 (7.4)	<0.001
SD-OCT[d]							
OPHF 3° temporal	37.86 (15.47)	16 (55.2)	13 (44.8)	26.36 (5.22)	21 (95.5)	1 (4.5)	<0.001
OPHF 6° temporal	31.41 (11.19)	22 (75.9)	7 (24.1)	28.45 (4.36)	21 (95.5)	1 (4.5)	0.15
OPHF 3° nasal	42.00 (23.11)	17 (58.6)	12 (41.4)	26.68 (5.87)	21 (95.5)	1 (4.5)	0.001
OPHF 6° nasal	35.34 (17.23)	24 (82.8)	5 (17.2)	29.14 (8.70)	21 (95.5)	1 (4.5)	0.13
ONL 3° temporal	55.86 (25.54)	17 (58.6)	12 (41.4)	73.18 (7.81)	21 (95.5)	1 (4.5)	0.002
ONL 6° temporal	52.93 (16.79)	21 (72.4)	8 (27.6)	58.36 (7.24)	21 (95.5)	1 (4.5)	0.11
ONL 3° nasal	52.21 (29.00)	16 (55.2)	13 (44.8)	74.59 (8.20)	21 (95.5)	1 (4.5)	<0.001
ONL 6° nasal	48.14 (17.96)	23 (79.3)	6 (20.7)	57.95 (10.33)	21 (95.5)	1 (4.5)	0.025

[a] *P* value from a linear mixed regression model testing for a difference between HCQ participants and normal control eyes on each measure of mfERG, MP, and SD-OCT.
[b] Average normalized response density for each ring.
[c] Mean sensitivity (dB).
[d] Thickness measurements (μm) of the Henle fiber layer (OPHF) and outer nuclear layer (ONL).

mfERG measures from equivalent locations (Supplementary Table S2). R2 and R3 showed a stronger correlation between mfERG and MP than measures from R1. Inferior partial rings showed stronger correlations between mfERG and MP than superior rings. The lack of correlation for R1 may be partly due to variability in the mfERG results associated with unstable fixation. By contrast, none of the SD-OCT thickness measures were significantly correlated with corresponding mfERG measures at equivalent retinal locations (data not shown).

Comparisons of mfERG, MP, and SD-OCT descriptive parameters between eyes from HCQ participants and normal eyes are displayed in Table 2. All mfERG rings except the superior R2/R6 partial ring were significantly worse on average in HCQ participants as compared to normal control eyes. The MP mean retinal sensitivity (dB) of all rings was significantly worse in HCQ-exposed eyes when compared to normal controls. With mfERG, 5 (10.9%) to 16 (34.8%) of the 46 HCQ eyes were classified as having abnormal function compared to 9 (19.6%) to 22 (47.8%) eyes by MP measures. Abnormal function was more frequent in the outer rings, namely, R2/R6 and R3/R6 of mfERG and MR2 and MR3 of MP. This trend was observed for

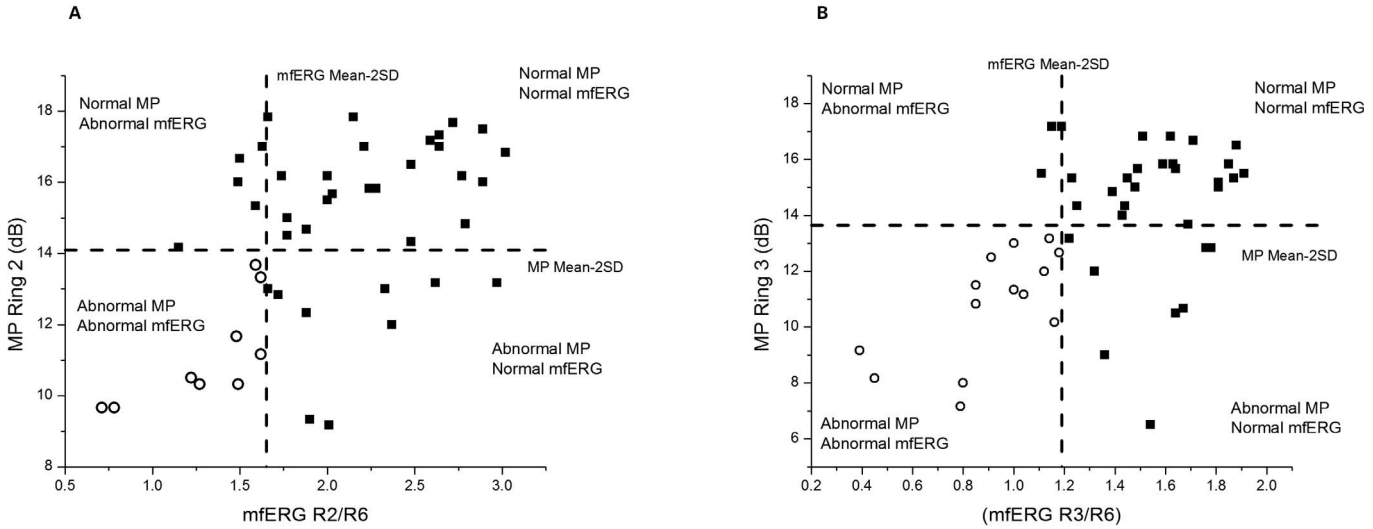

Figure 3. Scatter plot showing the distribution of normal versus abnormal measurements taken from HCQ eyes by both mfERG and MP in (A) ring 2 and (B) ring 3. The *dotted lines* represent the mean – 2 SD cutoff for determining abnormal function. *Open circles* represent eyes that were found to be abnormal by both mfERG and MP, and *closed squares* represent eyes that were normal by at least one measure.

both full and partial ring analyses. HCQ participants demonstrated greater impairment scores on MP measures than on mfERG measures relative to the normal mean in corresponding locations (Supplementary Figure S1). Significantly more impairment on MP compared to mfERG was found for full R2 and R3, partial superior R2 and R3, and inferior R3.

Using the mfERG as the reference test, we determined that 13 (28.3%) of 46 HCQ eyes were considered to have HCQ maculopathy according to the study criteria. Of the 13 eyes, 9 of 46 (19.6%) HCQ eyes also showed HCQ maculopathy based on MP. Of the 9 eyes that showed HCQ maculopathy by both mfERG and MP, 6 eyes showed abnormal OPHF and ONL measures where SD-OCT was available. Retinal sensitivity by MP was indicative of HCQ maculopathy in an additional eight eyes without concomitant abnormal function on the mfERG measures, and seven of these eight eyes did not show abnormalities on the SD-OCT measures.

For SD-OCT measures, diffuse HFL thickening[21] with relative shortening of the zone from the HFL to the ellipsoid zone in HCQ participants was the most common finding noted. There were also changes from control eyes in the photoreceptor interdigitation zone in the parafoveal region in 23 eyes, change in ellipsoid zone in 3 eyes, and perifoveal cupping in 6 eyes. Parafoveal SD-OCT changes were asymmetrical across and around the fovea. There was no significant difference in OPELM between HCQ and control eyes ($P > 0.05$, data not shown). However, OPHF was significantly thicker in HCQ eyes than in control eyes at 3° ($P < 0.001$) but not at 6° in the nasal and temporal retinas. The HCQ-exposed eyes also showed significant thinning of ONL compared to control eyes in all subfields ($P < 0.05$) except at 6° temporal (Table 2). Of the 29 HCQ eyes evaluated by SD-OCT, 12 (41.4%) to 13 (44.8%) were classified as having abnormal OPHF and ONL thickness at 3°, and between 5 (17.2%) and 8 (27.6%) were classified as abnormal at 6°. In general, SD-OCT measures were more likely to be abnormal at 3°, rather than at 6°, from the fovea. No obvious changes were noted in other retinal layers.

Sensitivity and Specificity of MP

Scatter plots showing the distribution of abnormal function by mfERG versus MP in HCQ participants are shown in Figure 3. In all cases except for comparisons of R1, more eyes were classified as abnormal based on MP measurements versus the corresponding mfERG measurements. The sensitivity and specificity of abnormal retinal function measured by MP relative to mfERG responses in corresponding locations is shown in Table 3. The sensitivity of full ring MP measures of abnormal function ranged from 33.3% (for MR1 versus mfERG R1/R6; CI, 13.8%–60.9%) to 87.5% (for MR3 versus mfERG R3/R6; CI, 64.0%–96.5%), with MR3 demonstrating improved sensitivity. Specificity ranged from 71.9% (CI, 54.6%–84.4%) to 85.3% (CI, 69.9%–93.6%), with MR1

Table 3. Sensitivity and Specificity of Microperimetry for Detecting HCQ Toxicity

MP Measure	mfERG Measure		Sensitivity[a] (95% Wilson CI)	Specificity[a] (95% Wilson CI)	*P* Value[b]
	Abnormal	Normal			
MR1	mfERG R1/R6				
Abnormal	4	5	33.3	85.3	0.41
Normal	8	29	(13.8, 60.9)	(69.9, 93.6)	
MR2	mfERG R2/R6				
Abnormal	9	9	64.3	71.9	0.29
Normal	5	23	(38.8, 83.7)	(54.6, 84.4)	
MR3	mfERG R3/R6				
Abnormal	14	8	87.5	73.3	0.06
Normal	2	22	(64.0, 96.5)	(55.6, 85.8)	
Superior MR2	mfERG Superior R2/R6				
Abnormal	5	15	100.0	63.4	<0.001
Normal	0	26	(56.6, 100.0)	(48.1, 76.4)	
Superior MR3	mfERG Superior R3/R6				
Abnormal	10	11	76.9	66.7	0.03
Normal	3	22	(49.7, 91.8)	(49.6, 80.3)	
Inferior MR2	mfERG Inferior R2/R6				
Abnormal	5	9	71.4	76.9	0.03
Normal	2	30	(35.9, 91.8)	(61.7, 87.4)	
Inferior MR3	mfERG Inferior R3/R6				
Abnormal	5	17	100.0	58.5	<0.001
Normal	0	24	(56.6, 100.0)	(43.4, 72.2)	

[a] Assumes that mfERG is the gold standard.
[b] McNemar's test for symmetry of abnormal determination between measures.

showing higher specificity. The sensitivity of partial ring comparisons ranged from 71.4% to 100%, and specificity ranged from 58.5% to 76.9%. The increased number of abnormally classified eyes by both MP and mfERG was statistically significant for the partial ring comparisons but not for the full ring comparisons (Table 3). MP superior MR2 and mfERG superior R2/R6 comparison and MP inferior MR3 and mfERG inferior R3/R6 exhibited significant discordance ($P < 0.001$), with 15 (32.6%) eyes and 17 (36.9%) eyes, respectively, being identified as abnormal by MP that were normal by mfERG, and no eyes (0%) identified as abnormal by mfERG but normal by MP. Overall, MR3 measures and partial rings showed fair to good sensitivity but decreased specificity in detecting functional deficits indicative of HCQ toxicity relative to mfERG measures.

Discussion

This study was performed to evaluate the potential utility of MP as a screening test for identifying HCQ-associated retinal toxicity and comparing it to the accuracy of the currently recommended mfERG screening test. By analyzing corresponding mfERG ring ratios and mean MP retinal sensitivities ring by ring at equivalent eccentricities from fixation, we demonstrated that MP categorizes more HCQ eyes as abnormal than mfERG.

The finding that MP had good sensitivity but was less specific for detecting abnormalities in our HCQ subject sample when classified against functional deficits observed on mfERG measures (Table 3) was true for full R3 and for the partial rings. Abnormal responses were identified from partial rings in some patients that were not identified as abnormal when full rings were considered. Thus, in cases where HCQ toxicity is suspected but not confirmed, partial ring analysis may be of benefit. In contrast to our findings, a recent study by Iftikhar et al.[12] on HCQ retinopathy showed MP to have good specificity (93%) and decreased sensitivity (73%) relative to mfERG. This disparity could be due to the use of differing mfERG ring ratios or differences in how abnormal function by

MP was defined (decreased mean ring sensitivity versus the presence of two or more contiguous abnormal points). Despite these differences, the sensitivity to detect HCQ toxicity for many rings in our study was similar to their sensitivity of 73%.

Currently, although the AAO recommends mfERG as a screening test when available, it does not yet have a position on standardizing the test protocol and method for analyzing mfERG data. mfERG has been used as a gold standard in some studies, but there are variations in the number of stimulus hexagons being used and the specific rings considered for analysis. For example, the R1/R2 ratio has been used for the evaluation of HCQ retinopathy.[8,12] The R1/R2 ratio may miss outer ring loss. Indeed, the present study, which used approximately matching coordinates between mfERG and MP and analysis of individual rings, showed a higher frequency of abnormal retinal function at R3 by both mfERG and MP.

Analysis by SD-OCT in HCQ eyes showed that thickening of the HFL, or thinning of the ONL, were considered to be hallmarks of abnormal structure. Because data were available for only a subset of HCQ eyes, more comprehensive analysis beyond the classification of measurements as normal versus abnormal relative to controls was not performed. Overall, using the study criterion for presence of HCQ maculopathy, we found that when both mfERG and MP measures were found to be abnormal, the SD-OCT was also abnormal. MP sensitivity was found to indicate HCQ maculopathy by the study criterion in eyes that were classified as normal by both SD-OCT and mfERG. However, in some cases eyes were classified as abnormal by SD-OCT when both mfERG and MP measurements were considered normal. Based on these results, MP may detect functional changes prior to structural changes on the SD-OCT, but further analysis and longitudinal studies are needed to make a formal determination. Other studies found that SD-OCT had clinically useful sensitivity and specificity relative to mfERG,[8] that SD-OCT exhibited decreased sensitivity and greater specificity relative to mfERG,[7] and that SD-OCT appeared to be less sensitive than MP in detecting retinal abnormalities.[6]

Unlike the mfERG, which only assesses retinal function, SAP and MP also detect contributions from components of the visual pathway further downstream from the photoreceptors, such as the inner retina. In a small case series of patients with chronic HCQ exposure, Pasadhika et al.[22] showed selective thinning of the inner retinal layers, particularly in the ganglion cell and inner plexiform layers in the absence of functional or structural changes in the photoreceptors and retinal pigment epithelium. However, a later study by de Sisternes et al.[23] found no changes in the inner retinal layers by SD-OCT segmentation in patients exposed to HCQ. Although we did not analyze the inner retinal layers by SD-OCT, MP may be useful for detecting early HCQ-associated inner retinal changes.

SAP, a recommended AAO screening test for HCQ toxicity, is done on the Humphrey visual field analyzer by using a 10-2 white stimulus and is widely used by clinicians. In clinical centers where the mfERG is not available, clinicians rely on the static visual field and the OCT to determine if HCQ toxicity is present. However, visual fields are highly variable,[8] and thus, interpretation of the static visual field test can be subjective if the reliability of the test is questionable. Similarly, OCT scans are interpreted qualitatively, which may result in overlooking subtle toxicity-related changes. An additional test, such as the MP visual field test, which can serve as a complement to SAP, may be done in place of the mfERG when the latter modality is not available. Although SAP and MP each have their advantages and limitations,[24] fundus-guided MP has the advantage of measuring visual sensitivity at discrete points on the retina with simultaneous tracking of the fundus. Due to the fact that MP is a subjective test and may be less specific than mfERG, it may be better suited as an initial screening test rather than a confirmatory or follow-up test. Although there are a limited number of studies utilizing MP to assess retinopathy in HCQ participants, this modality is being adopted as an effective outcome measure in various retinal conditions, such as retinal dystrophies,[25–27] diabetes,[28,29] and age-related macular degeneration.[30] The main limitations in our study are that SAPs were not available for all patients or had been done with either a white 10-2 or a red 10-2 or white 30-2 stimulus, thus precluding comparison of HVF and MP, and SD-OCT was available only on 17 (68%) of the HCQ participants.

In summary, we show that MP has good sensitivity to detect HCQ toxicity, although it is less specific and, thus, may be more likely to identify false positives. MP categorizes more subjects as abnormal than mfERG. Whether or not these abnormal characterizations are correct remains to be determined. It may be that early signs of toxicity are better detected with MP than with mfERG or SD-OCT, which could be investigated in a future longitudinal study. Neverthe-

less, the potential utility of MP as an alternative screening test should be further evaluated.

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