Multifocal Electroretinogram and Short-Wavelength Automated Perimetry Measures in Diabetic Eyes With Little or No Retinopathy

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Objective: To compare severity and locations of abnormalities detected by the multifocal electroretinogram (mfERG) and short-wavelength automated perimetry (SWAP) in diabetic eyes with early or no retinopathy.

Methods: One eye from each of 22 patients with diabetes mellitus who had early retinopathy and 18 patients with diabetes mellitus who had no retinopathy were tested on mfERG and SWAP. The mfERG implicit times were interpolated based on SWAP stimulus locations and compared with normative values obtained from 30 age-similar control subjects. The SWAP total threshold deviations were analyzed using an age-based control data set from 255 healthy subjects. The z scores of both measures were derived to allow measurement comparisons.

Results: Most responses for the 2 measurements were subnormal in both groups with diabetes mellitus. The 2 measurements showed a similar number of significant abnormalities (z score ≥2), about 40% and 20% of responses for diabetic patients with retinopathy and diabetic patients with no retinopathy, respectively. Local mfERG and SWAP results showed some spatial agreement for subjects with retinopathy (r=–0.38, P<.001) but not for those with no retinopathy.

Conclusions: Both mfERG and SWAP are sensitive measurements of diabetic dysfunction, even prior to retinopathy. The lack of spatial correspondence between mfERG and SWAP abnormalities in diabetic patients with no retinopathy reflects overlapping, but different, retinal anomalies in early diabetic eye disease.

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Diabetic retinopathy is the leading cause of blindness among working-aged people in the United States. Visual loss is generally irreversible at stages when non-perfusion regions, neovascularization, or both are clearly identified by ophthalmoscopy and fluorescein angiography. Early diagnosis, treatment, and prevention of retinopathy are essential to save sight.

The multifocal electroretinogram (mfERG) and short-wavelength perimetry (SWAP) are 2 promising measurements for early detection of visual functional changes in diabetes mellitus (DM). The mfERG is a powerful objective tool to study local retinal function, allowing us to simultaneously and independently record the cone-driven activity at more than 100 retinal locations within minutes. In eyes with diabetic retinopathy, local changes in retinal function are associated with the sites of retinopathic lesions. The mfERG detects functional abnormalities in patients with DM even before retinopathy is visible by fundus photography. The selective loss of short-wavelength (S-cone) visual pathway sensitivity has also been demonstrated psychophysically in diabetic patients with little or no retinopathy. The SWAP test, a subjective measure of local S-cone function across the retina, can identify the sensitivity loss in diabetic patients who have retinopathy and type 1 DM even without retinopathy.

Although both measurements are sensitive to diabetic retinopathy, they may provide different results because they are mediated by different cone systems. The mfERG responses reflect function of longer-wavelength-sensitive (L- and M-) cone pathways, while SWAP thresholds result from stimulating only the S-cone pathway. To date there has not been a direct comparison of the 2 techniques in the...
same patients. Since the 2 measurements reflect different mechanisms, one might anticipate little correspondence in the 2 measurements, or one measurement may be affected earlier than the other resulting in poor agreement.

This study has 2 aims. The first is to establish the extent to which the 2 noninvasive measurements, mfERG and SWAP, can detect functional loss in eyes with no or little diabetic retinopathy. The retinopathic lesions in our patients were much smaller than the stimulus sizes, distinguishing this study from previous mfERG or SWAP studies whose diabetic subjects had broader and/or advanced retinopathy. The second aim is to examine and compare the retinal locations and the retinal extent of the functional abnormalities detected by the 2 measurements.

**METHODS**

**SUBJECTS**

The left eyes of 22 patients with DM with early nonproliferative diabetic retinopathy (NPDR) (1 patient with type 1 DM and 21 patients with type 2 DM) and 18 patients with DM with no diabetic retinopathy (2 patients with type 1 DM and 16 patients with type 2 DM) were tested on mfERG and SWAP on the same day. Diabetic retinopathy was classified by a retinal ophthalmologist according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria.28 In the NPDR group, 2 subjects had moderate NPDR (each has a small patch of edema in the midperipheral retina); the other 20 patients with DM had only mild retinopathy. All eyes in both diabetic groups had 20/25 or better corrected visual acuity with refractive errors between –6.00 diopters and +4.00 D. Patients with visible media opacities or a history of other ocular disease or surgery were excluded from the study. The ages of NPDR subjects ranged from 32 to 59 years (mean age±SD, 52.4±6.0 years) with a duration of DM from 2 to 20 years (mean±SD, 10.2±6.2 years). The ages of the patients with no retinopathy ranged from 26 to 64 years (mean age±SD, 43.5±12.0 years) with a duration of DM from 3 to 20 years (mean±SD, 7.8±4.5 years).

Thirty eyes of 30 healthy (free of ocular or systemic disease) nondiabetic subjects were tested with the mfERG (19 right and 11 left eyes based on the subject’s preference). The 14 men and 16 women ranged in age from 28 to 60 years (mean age±SD, 47.2±9.5 years). All healthy eyes had 20/20 or better corrected visual acuity with refractive errors between –6.00 D and +4.00 D. Normal data for the SWAP test are based on 255 eyes of 255 healthy adults who ranged in age from 25 to 65 years (mean age±SD, 44.4±11.3 years; SAFE [Structure and Function Evaluation] study in Portland, Ore).29

The purposes and potential risks of the study were explained and informed consent was obtained from all subjects before testing. Procedures followed the tenets of the Declara-
tion of Helsinki, and the protocol was approved by the University of California Committee for the Protection of Human Subjects, Berkeley.

SWAP TESTING

The SWAP visual fields (Humphrey Field Analyzer; Humphrey Systems, Dublin, Calif) were tested with undilated pupils using the 24-2 stimulus presentation pattern (Figure 1A) and full-threshold strategy. The 24-2 pattern was chosen because its testing field closely matches the stimulus area of the mfERG responses. Three minutes of adaptation to a 100-candelas (cd)/m² yellow background preceded testing.25 An optimal lens correction was used, and the fellow eye was occluded with an eye patch. All of the subjects had fixation loss and false-positive and false-negative ratios less than 10%; most had ratios that were much less.

mfERG RECORDING

Multifocal ERGs were recorded using a stimulus-refractor unit (VERIS, version 4.3; Electro-Diagnostic Imaging, Inc, San Mateo, Calif). Pupils were dilated to 7 to 8 mm with a combination of 1.0% tropicamide and 2.5% phenylephrine hydrochloride. After the cornea was anesthetized with 0.5% proparacaine hydrochloride, a bipolar contact lens electrode (Hansen Ophthalmic, Solon City, Iowa) was placed on the test eye and a ground electrode clamped to the right earlobe. The fellow eye was occluded. The stimulus array of 103 hexagonal elements (Figure 1B) was delivered by an eye camera display refractor unit (Electro-Diagnostic Imaging, Inc) driven at a frame rate of 75 Hz. The hexagons were modulated between white (200 cd/m²) and black (< 2 cd/m²) according to a binary m-sequence during the 7.5-minute recordings. Before the test, observers adjusted the stimulus unit for best focus of the central fixation target. To improve the subject’s ability to maintain fixation, the test was broken up into 16 overlapping segments, each lasting approximately 30 seconds. The recording signals were filtered to 100 Hz and amplified 100,000 times. The quality of the recordings was controlled by a real-time display; eye movements were monitored by the eye camera. Contaminated segments were discarded and repeated. The mfERGs were processed in the usual way with 1 iteration of artifact removal and spatial averaging with 1/6 of the surrounding responses.

DATA ANALYSIS

Short-Wavelength Automated Perimetry

Based on the mean ± SD from healthy subjects of the appropriate age group at each testing location, z scores (standard deviation units in decibel domain) of total deviation were calculated for all diabetic participants. Total deviation represents the difference in decibels between the subject’s test results and the age-corrected normal values at each tested point in the visual field.

Multifocal Electroretinogram

A “template stretching” method described in detail by Hood and Li10 was used to measure the implicit time of the prominent peak (P1) of the first-order kernel (Figure 2). The 103 local mfERGs of each subject were compared with waveform templates representing the mean local waveforms of the healthy subjects (right eye responses were converted to left eye orientation). Each template was independently scaled in the amplitude and time dimensions so that the best least-square fit to each local response was obtained. Previous studies have shown that measurement of amplitude is relatively insensitive to DM and diabetic retinopathy.3,31,32 We also observed this in our study, therefore, herein we examined the relationship between SWAP and mfERG P1 implicit time measures.

Comparison of SWAP and mfERG Measurements

The spatial displays of mfERG and SWAP are different (Figure 1C). To compare the 2 measures point by point, one test’s results have to be interpolated to match the other. Herein we interpolated the 103 mfERG results based on SWAP stimulus locations because the mfERG has more testing points and, subsequently, provides more information for the interpolation (Figure 1D). In the Matlab program (The Mathworks, Natick, Mass), the mfERG results were interpolated into a high-resolution surface using a linear algorithm, and the interpolated mfERG value at each SWAP stimulus was determined by the coordinate of the SWAP stimulus.3,33 Because the extent of the testing field of the retina for the mfERG is slightly smaller than that of the SWAP field, the interpolated mfERG values beyond the actual testing area were not considered. The foveal test point was also excluded owing to the lack of availability of normal values at that location for the SWAP measurements. For the interpolated mfERG implicit times, at each location z scores were derived for each subject with diabetes based on the normal interpolated mfERG data. All further analyses use the z scores of the interpolated values.

RESULTS

SWAP AND mfERG RESULTS IN NPDR AND NO RETINOPATHY GROUPS

z Score distributions were constructed for both SWAP and mfERG measurements for the NPDR group from a
total of 704 values (22 subjects × 32 locations) and for
the no retinopathy group from 576 values (18 sub-
jects × 32 locations). The SWAP and mfERG z score
distributions in the no retinopathy group are shifted sig-
ificantly away from the theoretical normal distribution
(Mann-Whitney test, \( P < .0001 \) for SWAP; \( P = .0003 \) for
mfERG, Figure 3). As expected, both measurements
show that patients with NPDR tend to have more abnor-
mal findings than those with no retinopathy. In the NPDR
group, 38.6% of all SWAP z scores are −2 or less (re-
duced sensitivity) and 36.8% of the mfERG z scores are
2 or greater (implicit time delay, Figure 3A). For those
patients without retinopathy, although the distribu-
tions of the 2 measurements are closer to the healthy sub-
jects, 18.6% of SWAP z scores are −2 or less and 21.0%
of the mfERG z scores are 2 or greater (Figure 3B). These
z scores represent \( P < .02 \).

COMPARISONS BETWEEN SWAP
AND mfERG MEASUREMENTS

Are z-scores of SWAP and mfERG measurements lo-
caley spatially correlated across the retina? To answer this,
each retinal quadrant is examined (22 subjects × 8 re-
sponses per subject for the NPDR group and 18 sub-
jects × 8 responses per subject for the group without reti-
 nopathy). Figure 4 shows the results for the 2 diabetic
groups in the inferior nasal quadrant which is represen-
tative of the results of all quadrants. Most responses from
both measurements are worse than the average healthy

population in both diabetic groups (ie, most responses are
in the top left quadrant in each plot) despite the fact
that more than 90% of the locations had no detectable
retinopathy. The correlation coefficients between the 2
measurements were −0.38 for the patients with NPDR
\( (P < .001, \text{Figure 4A}) \) and −0.20 \( (P = .10, \text{Figure 4B}) \)
for the group with DM without retinopathy. The corre-
lation coefficients for superior temporal, superior nasal, and
inferior temporal quadrant are −0.38, −0.34, and −0.35 in
the NPDR group and −0.24, −0.23, and −0.21 in the group
with DM without retinopathy, respectively.

The correlation analysis we did above assumed a lin-
ar relationship between the 2 measurements, an as-
sumption that may or may not be correct. Therefore, we
next performed an agreement analysis, which assumed
no specific form for the underlying relationship be-
tween the 2 measurements. Two categories were de-
\( z \) scores at a specific location are both
defined, agreement and disagreement of the direction in
which both SWAP and mfERG measurements differ from
the mean of the healthy group (z score = 0) at each reti-

nal location. The 2 measurements are in agreement if the
SWAP and mfERG z scores at a specific location are both
or both better than the control means, and in dis-
agreement if one of the z scores is better and the other

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\begin{align*}
\text{Figure 3. Short-wavelength automated perimetry (SWAP) (A) and multifocal}
\text{electroretinogram (mfERG) (B) z score distributions for patients with}
\text{diabetes mellitus (DM). z Score distributions were constructed for the}
\text{patients with DM and early nonproliferative diabetic retinopathy (NPDR) from}
\text{a total of 704 values (22 patients from 32 locations) and for those with DM}
\text{without retinopathy from 576 values (18 patients from 32 locations). The}
\text{SWAP and mfERG z score distributions in the NPDR and no retinopathy}
\text{groups are shifted significantly away from the theoretical normal distribution}
\text{(shaded areas).}
\end{align*}
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is worse. By chance, we expect 16 of 32 common testing locations to disagree (ie, one measurement better than the control population, the other worse), and the remainder of the 16 locations to agree (ie, 8 better than the control population and 8 worse on both measurements).

For the NPDR group, however, SWAP and mfERG results agree on average at 30 (93.8%) of the testing locations (P < .001, Wilcoxon signed rank test) and very few of them are better than the healthy population (median values, Table). On the other hand, for the subjects with DM but no retinopathy, there is little correspondence between the 2 measurements (P = .12, Wilcoxon signed rank test). The number of locations where the 2 measurements disagreed is similar to our prediction (median values, Table). Although for both groups most responses are worse than those of the healthy subjects for each measurement, only in the NPDR group does the direction of the measurements disagree. SWAP and mfERG results agree.

Furthermore, the correspondence of the 2 measurements was examined when local responses were classified as normal or abnormal by a criterion z score of 2. For the NPDR group, 20.6% (145/704) of the responses were defined as abnormal by both SWAP and mfERG measurements at the same retinal locations, compared with only 8.7% (50/576) for the group with DM with no retinopathy. The abnormal results for mfERG and SWAP correspond considerably better for NPDR group than for the group with DM with no retinopathy.

Finally, we examined how well the 2 measurements could distinguish diabetic eyes from the healthy eyes. For each measurement an eye was classified as abnormal if more than 2 local responses were abnormal. This strict criterion is chosen on the basis of the following rationale. Since we chose z scores of ±2 as the criteria for abnormal results, each tested location has a 2.3% probability to be labeled as abnormal. As a result, based on the binomial distribution, the probability of more than 2 abnormal locations per eye is 3.7%. Therefore, classification of normal or abnormal eyes by this criterion makes it unlikely that a normal retina will be labeled as abnormal by chance. Nevertheless, we find that the SWAP test identifies 14 (63.6%) of the NPDR group and 9 (50.0%) of the group with DM with no retinopathy as having abnormal findings (Figure 5A) and the mfERG measurement classifies 13 (59.1%) of the NPDR group and 6 (33.3%) of the group with DM with no retinopathy as having abnormal findings (Figure 5B). The 2 measurements generally classified the same individuals as having abnormal findings; 11 eyes in the NPDR group and 5 eyes in the group with DM with no retinopathy are classified as having abnormal findings by both measurements.

COMMENT

Both the SWAP and mfERG implicit time measures show local functional abnormalities in the 2 groups of patients with diabetes we examined. More severe functional loss is seen in patients with DM with retinopathy than in those with DM with no retinopathy. There is a significant spatial correlation of the 2 measurements in the NPDR group. However, this is not evident in eyes with DM without retinopathy, consistent with the fact that the 2 measurements tap different but overlapping mechanisms.

Foveal measures have shown that the S-cone pathway is selectively susceptible early in diabetic retinopathy. In our study, the SWAP measurement, which probes S-cone pathway sensitivity across the extrafoveal visual field, is also affected in early diabetic eye disease. Previous studies using SWAP reported abnormalities in diabetic eyes with retinopathy, especially when...
SWAP defects have been previously reported in patients with diabetes mellitus (DM) (abnormality is defined as z scores ≤2 for the multifocal electroretinogram [mfERG] measurement and z scores of −2 or less for the short-wavelength automated perimetry [SWAP] measurement). Each dot represents the number of abnormal findings detected by SWAP or mfERG measurements for each patient with DM. The shaded areas indicate the number of abnormal findings per eye is less than 3. A, The SWAP results. B, The mfERG implicit time. NPDR indicates nonproliferative diabetic retinopathy.

In this study, the distribution of SWAP z scores in eyes with DM with no retinopathy is significantly different from healthy eyes, and 50% (8/16) of the eyes of patients with type 2 DM with no retinopathy have more than 2 local SWAP defects (and are, therefore, classified as being abnormal). The possible explanation is that the SWAP analysis we used considers the influence of age on ocular media (crystalline lens yellowing).20 Moreover, the larger normal database (n = 255) used may have sufficiently reduced the normal confidence interval so that SWAP sensitivity is significantly improved.20

In contrast with previous mfERG study findings of local retinal abnormalities in diabetic patients,5,6,31,32 the patients with NPDR have only mild diabetic retinopathy, and all of the visible lesion sizes are considerably smaller than the stimulus patches. Despite this minimal retinopathy, we find the mfERG implicit time to be a sensitive measurement of diabetic retinal function loss. We also find a high proportion of mfERG defects in diabetic patients who have yet to develop any retinopathy.

Previous studies provide some anatomical basis for the detection of mfERG abnormality in diabetic patients. Diabetic retinopathy is caused by defects of retinal capillaries lying mainly at the inner nuclear layer,36 various types of retinopathic lesion, such as microaneurysms, hard exudates, and retinal edema, occur in the middle layer of the retina, close to the inner nuclear layer. Pharmacological experiments on rhesus monkeys (Macaca mulatta) show that the major responses of mfERGs are generated by the bipolar cells,37,38 located in the inner nuclear layer of the retina.

In the NPDR group, mfERG and SWAP measurements at more than 90% of test locations agree in the direction of deviation from the normal mean (most toward abnormal). Thus, the 2 measurements agree well qualitatively for eyes with DM and retinopathy. However, the local correlation (r = −0.38), while highly statistically significant (P < .001), is quantitatively less robust. It could be the case that the 2 measurements would show better correlation if different scales of measurements were used.

Although both mfERG and SWAP measurements are sensitive to DM even in the absence of retinopathy, we found little local correspondence of the 2 measurements for patients with DM who have yet to develop retinopathy. For the eyes of patients with DM with no retinopathy, the 2 measurements do not agree qualitatively and they are not significantly correlated. This result might be related to the different mechanisms of response generation for the 2 measurements. The mfERGs mainly reflect L- and M-cone pathway activity prior to the nerve fiber layer. On the other hand, SWAP specifically tests the isolated function of the entire S-cone pathway, so abnormality can reflect disruption anywhere in the pathway. In the early stages of DM (prior to the retinopathy), the 2 systems may be differentially affected among different patients and/or retinal locations, resulting in the poor local correlation. However, after the development of diabetic retinopathy, all cone pathways might be affected. In this case both mfERG and SWAP are more likely to show abnormalities in the same or similar retinal locations.

It will be of interest to examine in the future whether SWAP or mfERG (or both) abnormalities in a particular retinal location might precede the development of retinopathy in that location. Our patients are participating in a 3-year longitudinal study that should reveal whether one test is a better predictor than the other or whether a combination of the 2 tests might have greater predictive power than either test alone.

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REFERENCES


