Formulation and Evaluation of a Predictive Model to Identify the Sites of Future Diabetic Retinopathy

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Purpose: To formulate and test a model to predict the development of local patches of nonproliferative diabetic retinopathy (NPDR), based on multifocal electroretinogram (mfERG) implicit times and candidate diabetic risk factors.

Methods: mfERGs and fundus photographs were obtained from 28 eyes of 28 diabetic patients during an initial and 12-month follow-up examination. mfERG implicit times were derived at 103 locations using a template-stretching method, and a z-score was calculated in comparison with 20 age-matched normal subjects. Thirty-five nonoverlapping retinal zones were constructed by grouping two to three adjacent stimulated locations, and each zone was assigned the maximum z-score within it. Zones containing initial retinopathy were excluded from further analysis. The probability that new retinopathy would develop in the remaining zones by the follow-up examination was modeled based on the mfERG implicit time z-score for the zone and other candidate diabetic risk factors determined during the initial visit. Data collected from four previously untested diabetic subjects and the other eye of eight previous subjects during their second year follow-up were used to test the predictive model.

Results: After 1 year, new retinopathy developed in 11 of the 12 NPDR eyes and 1 of the 16 eyes without initial retinopathy. After accounting for the correlation among zones within each eye, a predictive model was formulated with the variables mfERG implicit time, duration of diabetes, presence of retinopathy (NPDR or no retinopathy), and blood glucose level at initial visit. The area under the receiver operating characteristic (ROC) curve of this multivariate model is 0.90 (P < 0.001). The predictive model has an expected sensitivity of 86% and a specificity of 84%, which was verified by the test data.

Conclusions: The development of diabetic retinopathy over a 1-year period can be well predicted by a multivariate model. The inclusion of local mfERG implicit times allowed the model to identify the specific sites of future retinopathy. (Invest Ophthalmol Vis Sci. 2004;45:4106–4112) DOI:10.1167/iovs.04-0405

Diabetic retinopathy is one of the major complications of diabetes mellitus, a metabolic disease affecting 18 million people in the United States. Diabetic retinopathy ranks first among all causes of irreversible legal blindness in people of age 20 to 74 years. 1 The current clinical treatments for this eye disease successfully slow its progression but cannot fully reverse vision loss. Several studies have confirmed that clinical prognosis is better if patients are screened and treated early.2, 3

The prediction of the onset of diabetic retinopathy has been investigated in several studies. Most studies involved development of multivariate models to identify eyes with a high probability of future diabetic retinopathy based on the patients’ diabetic health information, including duration of diabetes, blood glucose level, cholesterol level, and presence or absence of microalbuminuria.4–7 Other studies attempted to improve the prediction by using additional visual function measures, such as blue-yellow color discrimination and oscillatory potentials recorded in conventional full-field electroretinograms.8–10 Although the specificity of these models is relatively high, the identification of eyes at risk of diabetic retinopathy is poor, with sensitivity below 60%.5, 6

A major limitation of these models is that the prediction of diabetic retinopathy is for the whole eye, not for specific areas of the retina. Diabetic retinopathy is a local disease, occurring nonuniformly across the retina. Therefore, if the specific sites of new diabetic retinopathy within an eye could be predicted, it might provide clinicians with a powerful tool to screen, follow-up, and even consider early prophylactic treatment of the retinal tissue in diabetic patients. Perhaps more important, the ability to quantify the risk of retinopathy would objectively strengthen future clinical trials of preventative pharmacological therapies.11–15

Recently, we identified a new risk factor for predicting local diabetic retinopathy: the abnormal multifocal electroretinogram (mfERG) response.15 The mfERG is a technique that can examine and map retinal function within 8 minutes at 103 locations in the posterior pole. It has been used to examine a variety of retinal diseases,16–18 including diabetic retinopathy.19–25 mfERG abnormalities have been reported in diabetic eyes, both with and without retinopathy. Furthermore, mfERG implicit time delays very frequently occur at sites of clinical lesions identified from fundus photographs in diabetic eyes with retinopathy.19–21 More interesting, as we reported, during a 1-year follow-up period, retinal patches with abnormal baseline mfERG implicit times are three times more likely to have new retinopathy than retinal patches with normal baseline mfERGs.14

The present study addressed three questions. First, could we formulate a quantitative model to identify sites of future retinopathy within an eye on the basis of mfERG implicit time? Second, could we enhance this model by adding additional diabetic risk factors (such as duration of diabetes and blood glucose level) to improve the predictive power for local retinopathy? Third, how well would the enhanced model perform when other diabetic eyes are tested?

To answer the first two questions, diabetic eyes with early or no diabetic retinopathy at baseline were re-examined, and areas with new retinopathy were identified 1 year after the initial study. The probability of development of local retinopathy visible on ophthalmoscopy was first modeled by baseline
TABLE 1. General Information for Diabetic Subjects

<table>
<thead>
<tr>
<th>Model making</th>
<th>Number of Subjects</th>
<th>Gender</th>
<th>Age (SD) (min, max) (y)</th>
<th>Diabetes Type</th>
<th>Duration of Diabetes (SD) (min, max) (y)</th>
<th>Blood Glucose (SD) (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPDR</td>
<td>12</td>
<td>Male: 7</td>
<td>52.5 (7.4)</td>
<td>Type 1: 2</td>
<td>10.2 (6.1)</td>
<td>191.2 (73.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female: 5</td>
<td>(30, 68)</td>
<td>Type 2: 10</td>
<td>(1.2, 19.1)</td>
<td>(100, 332)</td>
</tr>
<tr>
<td>No Retinopathy</td>
<td>16</td>
<td>Male: 7</td>
<td>50.1 (10.1)</td>
<td>Type 1: 3</td>
<td>5.2 (2.5)</td>
<td>146.2 (51.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female: 9</td>
<td>(36, 63)</td>
<td>Type 2: 13</td>
<td>(2.1, 10.1)</td>
<td>(84, 261)</td>
</tr>
<tr>
<td>Model testing</td>
<td>5</td>
<td>Male: 3</td>
<td>51.1 (10.1)</td>
<td>Type 1: 1</td>
<td>11.6 (8.2)</td>
<td>184.8 (73.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female: 2</td>
<td>(40, 64)</td>
<td>Type 2: 4</td>
<td>(2.2, 30)</td>
<td>(121, 273)</td>
</tr>
<tr>
<td>No Retinopathy</td>
<td>7</td>
<td>Male: 4</td>
<td>47.7 (12.0)</td>
<td>Type 1: 5</td>
<td>6.9 (4.0)</td>
<td>146.3 (53.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female: 3</td>
<td>(30, 62)</td>
<td>Type 2: 4</td>
<td>(1, 13)</td>
<td>(88, 236)</td>
</tr>
</tbody>
</table>

mFERG implicit time alone. Next, other diabetic risk factors were added to create a new model. Finally, the performance (sensitivity and specificity) of the final model was tested using data collected from 12 eyes that were not used in creating the models.

METHODS

Subjects

The predictive model was determined based on 1-year follow-up data from 28 diabetic patients (model-making group), including 12 with nonproliferative diabetic retinopathy (NPDR) and 16 without retinopathy. To test the model, another follow-up data set from 12 diabetic patients (model-testing group) was used. Four of them (one with NPDR and three without diabetic retinopathy) were new subjects whose data did not contribute to the creation of the model. Eight diabetic subjects (four with NPDR and four without diabetic retinopathy), whose data (on one eye) were used in formulating the model, were observed for one more year, and the second-year follow-up results (from the other eye) were used for model testing.

For all the diabetic subjects, at the first visit, medical history was collected and blood glucose levels were measured (Glucometer Elite meter; Bayer Corp., Elkhart, IN), and then mFERGs were recorded. Within 1.4 months of mFERG testing, dilated eye examinations were performed and stereoscopic color fundus photographs of the central 50° were taken. The 50° fundus photographs were chosen for this study, to cover the testing field of the mFERG, the central 45°. The 50° fundus photography is the standard protocol defined in the Field Guide Book and has been applied in the EURODIAB study. To normalize the mFERG measures, 20 eyes of 20 normal subjects (9 men and 11 women), aged 28 to 60 years (mean, 47.2 ± 9.5 years) were tested (14 right and 6 left eyes, based on the subject’s preference). All normal subjects were free of ocular and systemic disease and had 20/20 or better corrected visual acuity. No subjects with refractive errors outside the range of −6.00 to +4.00 D were included in this study.

The purposes and potential risks of the study were explained, and written informed consent was obtained from all subjects before testing. Procedures adhered to the tenets of the Declaration of Helsinki, and the protocol was approved by the University of California Committee for the Protection of Human Subjects.

mFERG Recording

mFERGs were recorded with a VERIS (Visual Evoked Response Imaging System, ver. 4.3; EDI, San Mateo, CA). Pupils were dilated to 7 to 8 mm with 1.0% tropicamide and 2.5% phenylephrine. After the cornea was anesthetized with 0.5% proparacaine, a bipolar contact lens electrode (Hansen Ophthalmic, Solon City, IA) was placed on the eye, and a ground electrode was clipped to the right earlobe. The fellow eye was occluded. An array of 103 hexagonal elements was delivered by an eye camera/display/refractor unit (Fig. 1A) driven at a 75-Hz frame rate. The hexagons were modulated between white (200 cd/m²) and black (<2 cd/m²) according to an m-sequence during the 7.5-minute recordings. Observers adjusted the stimulus unit for best focus of the central fixation target before the recording. Recordings were made in 16 30-second segments. Recording quality and eye movements were monitored by real-time display and the eye camera, respectively. Contaminated segments were discarded and repeated. Retinal signals were filtered 10 to 100 Hz and amplified 100,000 times. mFERGs were processed in the usual way with one iteration of artifact removal and spatial averaging with one sixth of the surrounding responses.

Data Analysis

mFERG analysis was similar to that used in our previous study. The first-order kernel local mFERG implicit times were measured using the template-stretching method described in detail by Hood and Li. Each template-stretching method described in detail by Hood and Li. The 103 mFERG stimulus elements were grouped into 35 zones (Fig. 1B). Two or three adjacent stimulated retinal patches were grouped approximately symmetrically across the test region. In this study, all subsequent data analyses were performed in a zone unit. We
A Local Predictive Model Based on mfERG Implicit Time Alone

We first considered the use of mfERG implicit time as the only risk factor for prediction of newly developed retinopathy at a specific retinal site. A GEE based on univariate logistic regression was used to establish a predictive model of development of retinopathy based solely on baseline mfERG implicit time. The equation for this model (where $Pr$ is the probability of development of new retinopathy in a zone, and the mfERG implicit time units are $z$-scores) is:

$$\ln\left(\frac{Pr}{1 - Pr}\right) = -2.84 + 0.21 \cdot mfergIT$$

The regression coefficient for this model (0.21) is significant ($P = 0.03$; Table 2). Longer mfERG implicit time ($mfergIT$) at a certain retinal location is associated with an increase in the probability of development of new retinopathy at the corresponding retinal location within 1 year. The odds ratio for $mfergIT$ in this model is $\exp(0.21) = 1.23$, which can be interpreted as an approximation of the relative risk, indicating that there is a 25% increase in the risk of new retinopathy associated with a unit change in the mfERG $z$-score.

To assess the accuracy of the model, the receiver operating characteristic (ROC) curve was calculated and plotted in Figure 2A. The ROC curve is a function of sensitivity versus 1 − specificity.

### Statistical Analysis

Logistic regression was performed to examine the association between the incidence of new retinopathy and seven risk factors: mfERG implicit time, duration of diabetes, age, gender, blood glucose level, diabetes type, and eye status (retinopathy present or absent at baseline). Because mfERG implicit times may be correlated among adjacent zones within an eye, generalized estimating equations (GEEs) were applied with corrected (robust) estimation of the variance−covariance matrix for model coefficient estimates that allows for this within-eye correlation.30 To estimate coefficients associated with each risk factor, a compound symmetric covariance structure was chosen that assumes a common covariance among the mfERG zones within an eye and independence between subjects.

### Results

Of the 980 (35 zones × 28 subjects) total mfERG zones in the model-making eyes, 61 (6.2%) had baseline retinopathy and were excluded from subsequent analysis. Fifty (82%) of the initial retinopathy signs were microaneurysms or dot hemorrhages, and 7 (11%) were hard exudates. One patch of edema and three patches of cotton wool spots occurred in the remaining four affected zones. Each retinopathy lesion was much smaller than one mfERG stimulus element. Over the 1-year study interval, new retinopathy developed in 11 of the 12 eyes with NPDR and in 1 of the 16 eyes without initial retinopathy. Excluding the mfERG zones with initial retinal lesions, new retinopathy at follow-up occurred in 64 (7%) of the remaining 919 mfERG zones. Fifty-seven (89%) of the zones with newly developed retinopathy contained microaneurysms or dot hemorrhages. The remainder were hard exudate (6 mfERG zones), cotton wool spot (2 mfERG zones), or edema (1 mfERG zone).

#### Table 2. Predictive Model Based on Univariate Analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>$P$</th>
<th>Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.57</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender</td>
<td>0.86</td>
<td>0.14</td>
</tr>
<tr>
<td>dmDuration*</td>
<td>&lt; 0.001</td>
<td>4.83</td>
</tr>
<tr>
<td>diabType*</td>
<td>0.58</td>
<td>0.62</td>
</tr>
<tr>
<td>bloodGlucose</td>
<td>0.39</td>
<td>0.01</td>
</tr>
<tr>
<td>mfergIT*</td>
<td>0.03</td>
<td>0.21</td>
</tr>
</tbody>
</table>

* Significant predictors.
A Local Predictive Model Based on Multivariate Analysis

In this section, we examine whether inclusion of known or suspected diabetic factors, which are not local predictors in themselves, enhances the local predictive power of the mfERG model. These factors were chosen because they are routinely obtained at each visit and do not require additional laboratory tests. The six variables were age, gender, diabetic eye status (with NPDR or without at baseline: basRet), duration of diabetes (dmDuration), blood glucose level at the initial visit (bloodGlucose), and diabetes type (type 1 or type 2: diabType).

The parameters age, dmDuration, and bloodGlucose were continuous variables on interval scales, whereas gender, basRet, and diabType were binary (yes/no) variables. In the analysis, female gender, diabetes with baseline retinopathy, and type 1 diabetes were defined as 1, and the counterparts of the three measures were defined as 0.

As is standard, we first examined the association of each variable alone with retinopathy development, using univariate logistic regression. Table 2 shows that whereas age, gender, diabType, and bloodGlucose were not significant predictors, dmDuration and basRet had significant power to predict the onset of new retinopathy \( P < 0.05 \). As expected, the regression coefficients for dmDuration and basRet were positive, indicating that a longer duration of diabetes or the presence of retinopathy at baseline, increased the probability of new retinopathy in the eye within 1 year.

Next, a preliminary multivariate model was established based on the variables that were shown in the univariate analysis to be significantly associated with the occurrence of future retinopathy: mfERG, dmDuration, and basRet. The \( P \) value for these variables in the preliminary multivariate model were 0.003, 0.109, and <0.001, respectively. These variables were included in the next stage of model building, because they all have \( P < 0.2 \). This criterion is chosen based on practical experience that a variable with \( P < 0.2 \) provides some predictive power without adding a significant amount of variation. Variables bloodGlucose, age, gender, and diabType, which were not significant predictors in the univariate analyses, were added back to the preliminary multivariate model one at a time, in the order of their \( P \) value in the univariate analysis, from low to high (i.e., stronger factors first) to assess their additional contributions.

Age \( (P = 0.75) \), gender \( (P = 0.96) \), and diabType \( (P = 0.61) \) did not provide significant information to the model prediction, and so they were excluded from the final model, whereas the variable bloodGlucose \( (P = 0.17) \) met our criterion of \( P = 0.2 \), and was included. Our final estimated multivariate model to predict the local sites of retinopathy was formulated as

$$
\log \left( \frac{Pr}{1 - Pr} \right) = -6.78 + 0.32 \cdot mfERG + 3.84 \cdot basRet + 0.14 \cdot dmDuration + 0.005 \cdot bloodGlucose.
$$

The odds ratios for all the variables in the final model were greater than 1 (Table 3, column 4), indicating that the variables all correlated positively with the development of new retinopathy, though the odds ratio of variable bloodGlucose does not reach significance. With all other variables fixed, the odds ratio of development of new retinopathy is 1.15 for each 1-year increase in duration of diabetes (dmDuration). The corresponding odds ratio for 5-year increment in duration is 2.01 (95% confidence interval: 1.09–3.70). The odds ratio for variable basRet is large (46.4), suggesting that the presence of diabetic retinopathy is a strong predictor of future retinopathy in diabetic persons, even in a short period. However, caution
should be used, given its large confidence interval. After adjustment for the other variables, the local predictor, mfergIT is still significant, with an odds ratio of 1.38 for a unit increase in the mfERG implicit time z-score.

Figure 2B shows the ROC curve for the final model, which has an AUC of 0.90 (SE = 0.008; P < 0.001) compared with an AUC of 0.80 obtained from the model with mfERG only. For a cutoff of Pr = 0.4, the model provided a sensitivity of 86% and a specificity of 84%, which are substantially higher than the model with mfergIT as the sole predictor, reflecting that the final multivariate model more accurately predicts new diabetic retinopathy at specific retinal sites.

Testing the Local Predictive Model

As described in the Methods section, 4 of the 12 diabetic subjects (1 with NPDR and 3 without) were used to test the final model were new subjects. Eight (four subjects with NPDR and four without) were subjects in the model-making group whose model-testing data were collected from the other eye at the second follow-up. At baseline (the first visit for the four new subjects and the second visit for the rest), 10 (2.3%) of the 420 zones, corresponding to a specificity of 85.7% (95% confidence interval: 82.1%–89.3%; Table 4).

Testing of the Predictive Model

In this study, we developed a predictive model to identify the sites of future diabetic retinopathy over a 1-year period, based on the data measured from more than 900 retinal patches (28 eyes). This model calculated the probability of new retinopathy, depending on mfERG implicit time, duration of diabetes, diabetic eye status, and blood glucose level. With a cutoff of P = 0.4, it successfully discriminated normal retinal locations from those where new retinopathy had developed 1 year later, with a sensitivity of 86.0% and a specificity of 84.0%. In addition, the model was tested using data collected from four new diabetic subjects and the other eye of eight model-making subjects during their second-year follow-up. The test sensitivity and specificity were 89.4% and 85.7%, respectively, very close to our expectation.

Risk factors, such as duration of diabetes and blood glucose level, which are known to predict the eyes with risk of future retinopathy, were shown in this study to strengthen the local predictive power of the mfERG implicit time. Duration of diabetes is known to be one of the major risk factors. The Wisconsin epidemiologic study reported that only 27% of patients who had had insulin-dependent diabetes mellitus for 5 to 10 years had diabetic retinopathy compared with 71% to 90% of those who had had diabetes for longer than 10 years. The result in patients with non-insulin-dependent diabetes mellitus is slightly different, though the incidence of diabetic retinopathy and the duration of diabetes also correlate positively. Ten years after the diagnosis of type 2 diabetes, 67% of patients had retinopathy.

Another known risk factor is blood glucose control. The Diabetes Control and Complications Trial (DCCT) showed that diabetic patients with tight control (defined as three or four insulin injections per day) had a 76% decrease in the rate of development of any retinopathy and an 80% decrease in the progression of existing retinopathy compared with those with loose control (one or two insulin injections per day). Although blood glucose level is not significant either in our univariate or multivariate analysis, including it increases the predictive strength slightly (5%), according to the area under the ROC curve. Perhaps if glycated hemoglobin, a measure of average blood glucose over the preceding 3 months, were used, a stronger association with development of retinopathy would be identified. Alternatively, it has been suggested that fasting blood glucose levels, or the level of plasma blood glucose 2 hours after loading, have equivalent predictive power to HbA1. These other measures generally require additional laboratory facilities.

Our results also indicate that existence of diabetic retinal lesions is a significant risk factor for the occurrence of future retinopathy. Eleven of 12 diabetic subjects with NPDR at baseline had new retinopathy at follow-up compared with only 1 of 16 with no retinopathy at baseline. The low conversion rate from no retinopathy to NPDR accounts for the wide 95% confidence interval for variable basRet, making the final model more robust for the prediction of future retinopathy in diabetics with retinopathy than in those without it. Additional data

**Table 4. Testing of the Predictive Model**

<table>
<thead>
<tr>
<th>Retinopathy</th>
<th>New Retinopathy</th>
<th>Still No Retinopathy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy predicted by model</td>
<td>42</td>
<td>52</td>
<td>94</td>
</tr>
<tr>
<td>Normal location predicted by model</td>
<td>5</td>
<td>311</td>
<td>316</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>363</td>
<td>410</td>
</tr>
</tbody>
</table>
on a larger number of “converters” would improve the precision of this estimate.

mfERG implicit time was the only variable in our model that could identify local retinal sites where new retinopathy occurs. The primary generators of the mfERG, bipolar cells, are located close to where diabetic retinopathy, such as microaneurysms, hard exudates, and retinal edema, occur, which implies that bipolar cell function is abnormal by the time or even before retinopathy is present. We believe that this is one of the possible reasons that the mfERG is sensitive in diabetes and diabetic retinopathy.

The present study extends findings in our earlier study, which established mfERG implicit time as a risk factor for development of future retinopathy, in four important ways: First, it offers a quantitative model that can discriminate whether retinopathy will or will not develop in a retinal patch within 1 year with good accuracy (86% sensitivity and 84% specificity), rather than just specifying the relative risk of retinopathy, as in our earlier study. Second, the GEE method was used to perform logistic regression, thereby taking into account the correlation among the mfERG zones, improving the accuracy of model fitting. Third, this predictive model applies mfERG implicit time as a continuous variable rather than a binary categorical variable (normal or abnormal according to a fixed z-score criterion). In this way, we can specify the amount of risk corresponding to a given increment change in mfERG implicit time z-score unit. Fourth, smaller mfERG zones were used, two to three stimulated areas in this study compared with up to seven elements in our previous study, providing more localized prediction.

The model was tested on a limited basis. The sensitivity and specificity from the testing data were high and similar to our expectations. Although many of the model-testing subjects were also in the model-making group, the testing data were collected from the other eye in the subsequent follow-up period, minimizing the possibility that our estimates of sensitivity and specificity are overly optimistic. In the future, testing the model with a data set collected from an independent and larger population will provide more objective assessment of the predictor.

To our knowledge, this study is the first to formulate a quantitative model to predict the specific sites of future diabetic retinopathy in a 1-year period. In the future, we will examine whether the predictive power of the model can be further improved by including additional local measures that are sensitive to diabetic visual function loss, such as short-wavelength automated perimetry or other applications of the mfERG, and we will examine predictive ability over different time intervals after mfERG measurement.

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References


