Original Article

THE USE OF MULTIFOCAL ELECTRORETINOGRAM TO PREDICT PROGRESSION OF DIABETIC RETINOPATHY

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ABSTRACT

The primary cause of visual loss in diabetic retinopathy (DR) is macular edema. Predicting the occurrence of diabetic macular edema may allow institution of early treatment in diabetic patients. A prospective observational study was conducted to determine whether abnormal implicit time in multifocal ERG or mfERG (mfERG IT) within the macular region can predict progression of DR after one year. A total of fifty patients with type 2 diabetes and mild to moderate non-proliferative diabetic retinopathy (NPDR) was utilized. At baseline, patients' mfERG from 61 retinal points within 35 degrees from the center of fovea were recorded and fundus photographs were taken at baseline and 12 month. mfERG IT at baseline were measured and fundus photograph were used to monitor progression of DR within 1-year. The result revealed that 1552 retinal points with abnormal mfERG IT showed DR progression after 1 year. Relative risk of DR progression among retinal points with abnormal mfERG IT at baseline were 6 times greater than retinal points with normal mfERG IT (RR 6.21; p < 0.001). mfERG IT at baseline has 89.9% sensitivity and 81.7% specificity to predict progression of DR. In conclusion, abnormal mfERG IT provides an objective assessment of local retinal health in diabetes and may be useful to predict DR progression.

INTRODUCTION

The prevalence of Diabetes Mellitus (DM) is expected to increase two- fold from 2.8% or 171 million population in the year 2000 to 4.4% or 366 million population in 2030 [1]. In10.2 million adults aged 40 years and older with DM, the estimated crude prevalence rates for retinopathy and vision threatening retinopathy were 40.3% (4.1 million person) and 8.2% (836,400 person) respectively [2]. The primary cause of visual loss in diabetic retinopathy (DR) is macular edema, caused by leakage from micro aneurysms and dilated capillary segments. Diabetic macular edema (DME) can occur at any stage of DR and may result in devastating visual complication. Therefore, predicting and preventing macular edema in "at risk" individuals would be of great benefit for diabetic patients.

Recently, multifocal ERG (mfERG) has emerged as a new technique for exploring human retinal function including DR [3]. Several studies have shown that mfERG implicit times (IT) were significantly increased and the mfERG amplitude (Amp) were significantly reduced in diabetic patients with or without retinopathy [3,4]. Other relevant study reported that the relative risk (RR) of developing new DR over one year in the areas with abnormal mfERG IT was approximately 21 times greater than in eyes with normal baseline mfERG IT [4]. Nevertheless the study only involved a small number of subjects and used larger retinal zones rather than small unit of retinal points.

The purpose of this study is to assess whether abnormal mfERG IT within the macular region at baseline are predictive of new development or worsening of DR after 12 months and to study the correlation between the number of retinal points with abnormal mfERG IT that has developed new or worsening DR after 12 months with glycosylated hemoglobin (HbA1c) level, duration of diabetes and diabetic treatment.

METHODOLOGY

Subjects

This was a prospective observational study among type 2 diabetic patients with mild to moderate

NPDR. This study was conducted at the Department of Ophthalmology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia. The study followed the tenets of the Declaration of Helsinki and was approved by the UKMMC Ethics Committee for protection of Human Subjects. Patients with only mild to moderate NPDR were included in the study. All subjects were free from other ocular diseases apart from DR and had the best corrected visual acuity (BCVA) of 20/20 or better.

Baseline

At baseline, all patients had their visual acuities measured using the Snellen chart, a complete dilated ocular examination using the slit lamp and blood taken for HbA1c level. Fundus photographs using Topcon TRC 50DX Type 1A Retinal Camera were obtained. The macular area was determined by an imaginary circle of which the radius is a distance between the center of the macular and including the temporal 1/3 of the optic disc. Only one eye with the clearest fundus image was included in the study. The images were cropped and printed out using A4 size glossy paper.

MfERG procedure

After 15 minutes exposure to ordinary room light (recovery period), mfERG examination were performed using the Espion mfERG by Diagnosys, LLC. The selected eye was anesthetized with topical instillation of Proparacaine Hydrochloride 0.5% and the patient's temporal part of the scalp, midline of the forehead and both sides of the nasal bridge were cleaned with alcohol swab and scrubbed using the skin prep paper. The Dawson-Trick-Litzkow (DTL) fibers were then placed on the inferior fornices of the tested eye. The fibers were held by two skin electrodes, one placed on the temporal scalp and the other on the sides of the nasal bridge. A ground electrode was placed on the midline of the forehead and an eye occluder covered the untested eye

The patient was then seated comfortably 30 cm from the LCD screen with their head placed in the built-in headrest. The 61 hexagons 35 degree protocol was used for this study which was an array of 61 hexagonal elements delivered within a field of 35 degrees. During stimulation, the displays appeared as a flicker of each hexagon that will go through a binary m- sequence of black (Lmin = 0cd/m^2) and white (Lmax = 1000cd/m^2). In order to increase the reliability of the test, blink rejection was set up to 20µV and noise rejection was set up to 50µV to filter the unnecessary waveforms produced by noise and blinking reflex. A reliability index of 98 -100% was aimed for each test. To increase patient concentration, the test was paused and re- run repeatedly to allow the tested eye to rest for a few seconds. The overall test took about 8- 10 minutes to complete. In this study, a standard algorithm of m- sequence which was recommended by the International Society of Clinical Electrophysiology of Vision (ISCEV)standard for clinical mfERG (2011 edition) was used [5]. The m-sequence controls the sequences of change between light and dark stages of each hexagon. This caused each hexagon to change with every different frame. The mfERG test was repeated twice and the mean from all three readings were taken.

At the end of the test, the trace array, topographic 3-D density plots and concentric rings averages were obtained. In trace array, the smoothed (filtered) rather than raw data was used. This smoothed data eliminated surrounding noise and thus further smoothed the raw data.

Trace array MfERG result were printed on an A4 size overhead projector transparent paper and superimposed on the printed A4 size color fundus photo on high quality glossy paper. This was used as the reference point during the patient's follow up at 12 months.

Follow up

At 12 months follow up, fundus photographsof the study eye was repeated. The images were cropped around the area of interest for analysis and printed out. The trace array mfERG at baseline on the OHP transparent papers were then superimposed on the 12 months printed color fundus photo.

Outcome measures

The mfERG IT was defined as the time from the beginning of the wave until the first prominent response peak (P1). Abnormal mfERG IT was defined as mfERG IT longer than the normal IT of the local population normative database.

Each of the retinal points were classified into different outcomes which included true positive (TP), false positive (FP), true negative (TN), and false negative (FN). True positive was defined as retinal points with abnormal mfERG IT at baseline and the DR progressed. False positive was defined as retinal points with abnormal mfERG IT without DR progression. True negative was defined as retinal points with normal mfERG IT and no DR progression and false negative was defined as retinal points with normal mfERG IT that progressed. The definitions of DR progression and no DR progression are illustrated in Figure 1 and Figure 2. In order to reduce bias in the results, mfERG at baseline were analyzed by the primary investigator, and fundus photo at baseline and month 12 were analyzed by 2 different medical retina experts.

Data analysis

Retinal points with TP, FP, TN, and FN results were summed up. The mean of mfERG intrinsic time between TP and FP, TN and FN were analyzed using independent T- test. The area under curve (AUC) and relative risk (RR) for the development of new or worsening DR in the macular region were calculated.

RESULTS

Patients' characteristics

A total of 50 eyes from 50 participants were included

in this study. The mean age was 59.5 ± 8.3 years (range 42- 77). There was a slight female preponderance with the male to female ratio of 1: 3. There was no racial predilection (p= 0.737). The over-all mean duration of diabetes was 12.1 ± 7.0 years (range 2- 32 years). The mean HbA1c of all 3 months was 8.4 ± 1.76 mmol/ml.

Implicit times and development of DR

The mean number of retinal points with abnormal mfERG IT at baseline that had DR progression after 12 months i.e TP were found to be significantly more (31.06 retinal points, p< 0.001, 95% CI 23.20- 29.32) compared to mean number of retinal points that did not showed progression DR despite an abnormal mfERG IT at baseline i.e FP. In addition, the mean number of retinal points with normal mfERG IT at baseline that did not showe DR progression i.e TN were significantly more (21.40 retinal points, p< 0.001, 95% CI 15.52-20.68) compared to mean number of retinal points which showed DR progression despite a normal mfERG IT at baseline i.e FN (3.48 retinal points). Implicit times provide good accuracy (p< 0.001, AUC = 0.89, 95% CI 0.87-0.90) in predicting DR in 12 months. (Figure 3) Based on the calculation, mfERG IT at 38.40ms is a cut off point to predict DR progression. Our study also found that retinal points with abnormal mfERG IT at baseline were found to be 6 times more likely to develop DR in 12 months compared to retinal points with normal mfERG IT(RR= 6.23, 95% CI 5.43 – 7.17). The sensitivity and specificity of mfERG IT to predict DR progression was 89.9% and 81.7% respectively (Table 1).

DISCUSSIONS

This study was aimed to see whether abnormal mfERG IT within the macular region at baseline are predictive of Diabetic Retinopathy progression after 1 year. This is a large scale study involving 3050 retinal points from 50 subjects. This study demonstrated that abnormal mfERG IT may be useful in determining DR progression.

Patient characteristics

The mean duration of diabetes among patients within the study is 12.1 ± 7.0 years with the shortest duration of 2 years and the longest duration of 32 years old. This was consistent with other studies that showed the estimated onset of detectable retinopathy is between 4 to 7 years before the diagnosis type 2 DM. Duration of DM type 2 has been proven to be a strong risk factor for DR progression [6,7,8,9].

Abnormal mfERG IT and the progression of DR

This study highlighted that mfERG IT has a strong predictive value in predicting the development and progression of DR. mfERG IT also has a high sensitivity (89.9%) and specificity (81.7%) in predicting DR progression. We also found that retinal points with abnormal mfERG IT is 6 times more at risk of developing new or worsening of DR within 1 year. The mfERG IT measurements can be used to monitor the progression of DR during the early stages of disease and can also evaluate the effectiveness of preventative drug therapies that are currently being developed [4].

Interestingly, these results also match those observed in an earlier study that demonstrated that implicit times rather than the amplitude of mfERG was more sensitive in assessing retinal function in diabetics than amplitude mfERG [10,11]. Over a 1 year period, mfERG IT increased in most of the retinal areas of eyes with NPDR but remained constant in normal subjects and in diabetic patients without retinopathy [4]. In contrast, amplitude mfERG were not associated with DR and did not predict retinopathy.The reason for prolonged implicit times prior to the occurrence of diabetic lesions has been described by Cao et al.[12]. Prior to visible fundus lesion, pericyte apoptosis and basement membrane thickening results in the occurrence of acellular capillaries indicating early vasculopathy in diabetic eyes. Four major theories have been proposed to explain how chronic hypoglycemia and subsequent retinal hypoxia might lead to these anatomic changes: 1) increased formation of advanced glycosylation end products; 2) abnormal by pass of glucose metabolism through the sorbitol pathway; 3) activation of growth factors such as the vascular endothelial growth factor; and 4) oxidative stress and free radical generation which promotes the development of diabetic lesions. These compromised local metabolisms affect the functions

Table 1: Analysis between implicit times mfERG and progression of diabetic retinopathy

		p	95% CI
RELATIVE RISK	6.32		5.41- 7.19
SENSITIVISITY (%)	89.9		
SPECIFICITY (%)	84.2		
AREA UNDER CURVE (AUC) (%) OF ROC CURVE	0.89	<0.001	0.86- 0.90
BEST CUT OFF POINT (ms)	38.40		

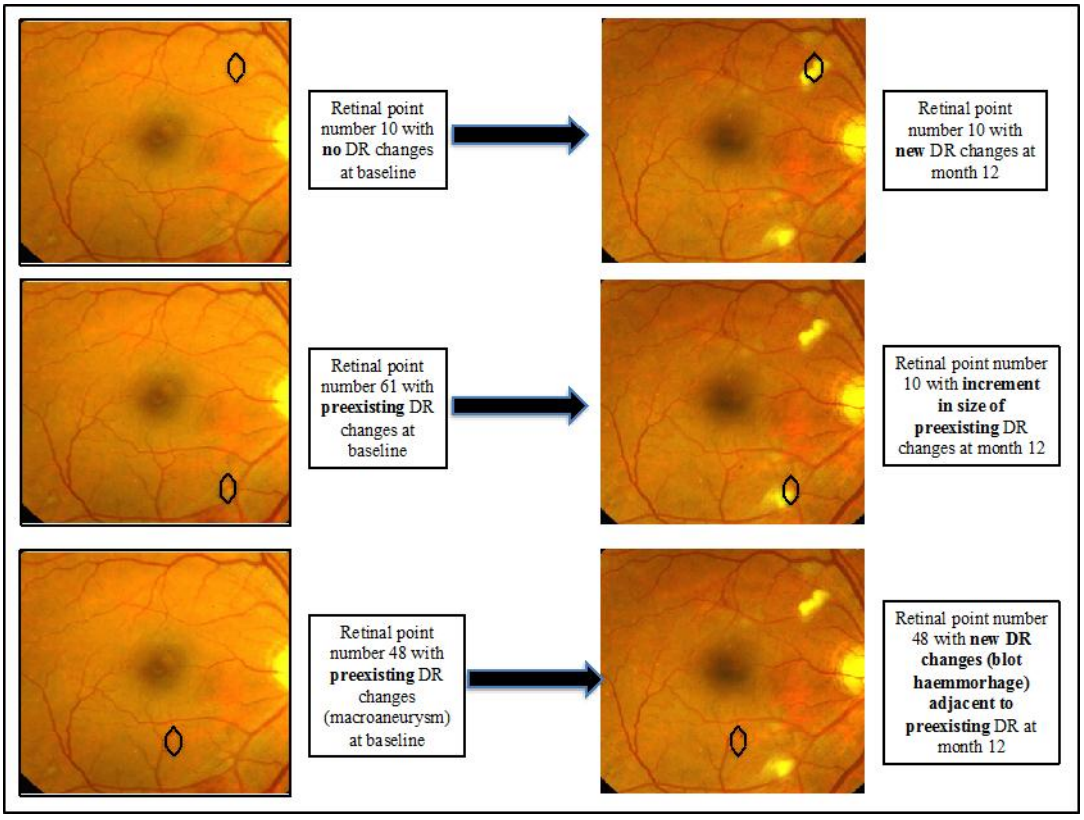

Figure 1: 35 degree fundus photograph at baseline (left) and at month 12 (right) showing DR progression

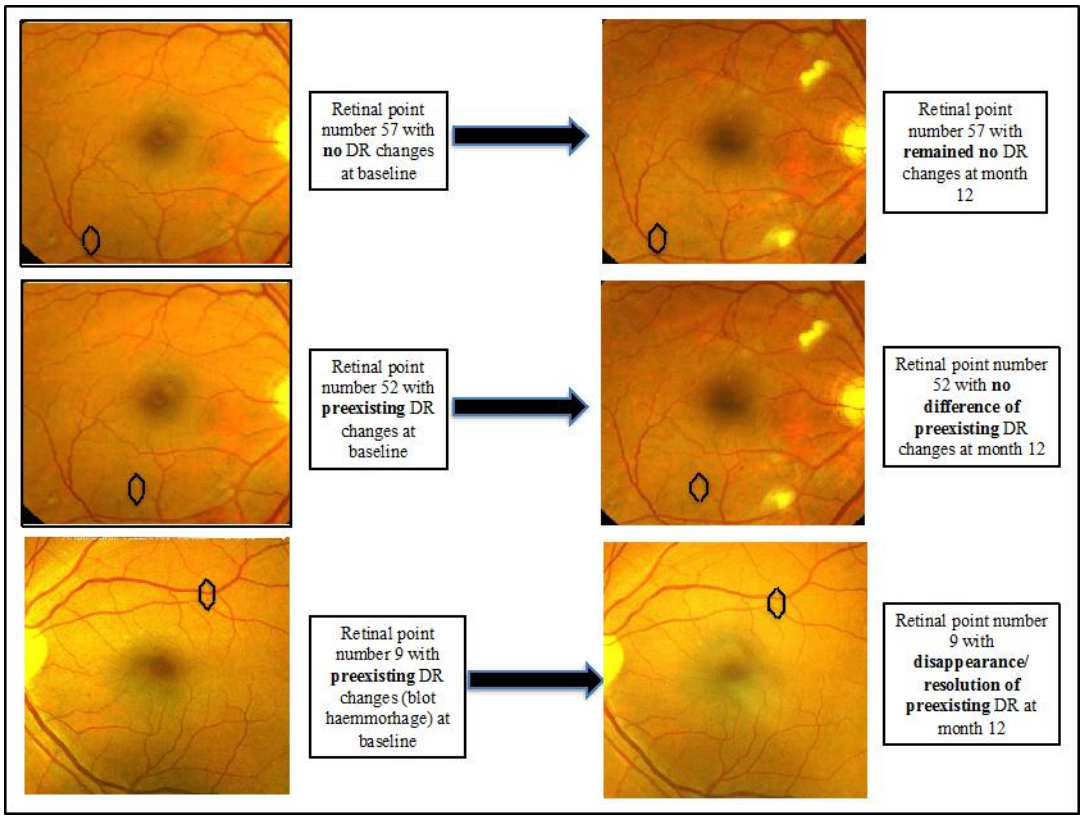

Figure 2: 35 degree fundus photograph at baseline (left) and at month 12 (right) showing no DR progression

of mfERG generators (photoreceptors and bipolar cells), leading to delayed neural conduction and prolonged mfERG IT, subsequently resulting in an abnormal mfERG reading despite normal retinal appearance.

Fortune et al has demonstrated that the presence of significant delayed local response in mfERG among retinal points without retinopathy is a very early indicator of local retinal dysfunction in diabetes [13]. It is possible that early local mfERG delays, found in the absence of retinal vascular findings are caused by early diabetic choroidal lesion. Retinal hypoxia is thought to be a major stimulus leading to increased expression of vascular endothelial growth factor (VEGF) [14]. In turn, increased expression of VEGF is likely to be a critical factor in the development of even the earliest retinal vascular lesion in diabetic retinopathy.

This study also found that mfERG is well suited in the study of DR. This is based on the fact that, DR is a retinal disorder with localized lesion typically confined to the posterior pole, the same site where standard mfERG tests local retinal function (across 35 to 45 degree). Secondly, DR is largely caused by defects of retinal capillaries in the inner nuclear layer of the retina, where bipolar cells' cell bodies, the primary generators of the mfERG are located [15]. Taken together, these results suggest that the mfERG IT could play an important role for monitoring local metabolic condition in diabetes.

In conclusion, this study demonstrated that abnormal mfERG IT has a good predictive value for DR progression. The mfERG provides a very sensitive and objective assessment tool for local retinal health in diabetes and would benefit in the assessment and management of DR in the future. Further future work may be performed to show whether mfERG IT could also predict the development of DR in eyes without retinopathy. Concurrent use of other modalities such as the OCT and FFA in the assessment of macular area would indeed give a more accurate anatomical macular assessment in relation to mfERG.

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Conflict of Interest
No conflicting relationship exists for any author.

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