Role Of Multifocal Electroretinography In Assessing Local Retinal Abnormalities In Diabetic Subjects With And Without Retinopathy

Jagdeep Kaur S. Dani*, Archana H. Patel, Mitesh M. Sinha**, Geeta B. Nair***, Sima K. Bhatt***

*Associate Professor, M & J Western Regional Institute of Ophthalmology, Ahmedabad, 380016,

** Resident***Asso. Professor, Department of Physiology, B. J. Medical College, Ahmedabad, 380016

Abstracts: Background: Electrophysiological studies of humans with diabetes could be used to assess alterations such as dysfunction of ganglion cells and loss of colour and contrast sensitivity. Sensitivity of the full-field ERG is limited, precisely because it reflects the activity of the entire retina. In contrast, mfERG the ability to measure local ERGs in diabetes which would improve objective detection of early functional alterations and assessment of local change over time. Aim: The purpose of this study was to identify local retinal abnormalities in diabetic subjects with and without non-proliferative diabetic retinopathy (NPDR) using the mfERG. Method: This is a cross sectional study of patients who were clinically diagnosed and sent to us by M& J Regional Institute of Ophthalmology, Ahmedabad, India. Subjects were divided into three groups as group A (normal), B (Diabetics without Retinopathy) and C (Diabetics with Retinopathy) on the basis of medical/ocular history, visual acuity and fundus. mfERG was done in all subjects and the responses were analysed using t test. Result: P1 response density was decreased in Group B and Group C subjects as compared to control in Group A. In subjects with diabetic retinopathy the N1 latencies were higher as compared to diabetics - no DR and control group. When compared the mean global P1 implicit time increased from normal to diabetics - no DR group and DR group. Conclusion: The presence of significant local response delays in eyes without clinically evident retinopathy suggests that such mfERG changes may provide a very early indicator of local retinal dysfunction in diabetes.

Key Words: Diabetic Retinopathy, mfERG, N1 Latency, P1 Implicit time

Author for correspondence: Dr. Jagdeepkaur S. Dani, Asso. Prof. And Head of Occular Physiology, M & J Western Regional Institute of Ophthalmology, Ahmedabad.e- mail: dr.jagdeepdani@gmail.com

Introduction: Retinopathy is the most common microvascular complication of diabetes, and it remains a major cause of visual impairment worldwide. Almost all patients with type 1 diabetes will develop retinopathy over a 15 to 20-year period, and approximately 20-30% will advance to the blinding stage of the disease. More than 60% of patients with type 2 diabetes will have retinopathy.[1]

Vascular lesions in the early stages of diabetic retinopathy are characterized by the presence of capillary microaneurysms, pericyte deficient capillaries, and obliterated and degenerated capillaries. Proliferative diabetic retinopathy is the more advanced form of the disease, when circulation problems cause the retina to become oxygen deprived. As a result, new fragile blood vessels can begin to grow in the retina and into the vitreous. Therefore, diabetic retinopathy has long been recognized as a vascular disease. The neuronal cells of the retina are also affected by diabetes.

Electroretinography (ERG) is the neurophysiological test used in order to measure electric changes that happen in the retina after a light stimulus. Changes in the ERG may be due to impairment of any of the retinal cell types: photoreceptors (a-wave ERG), and amacrine, bipolar, and, mainly, Muller cells (b-wave ERG). Moreover, oscillatory potentials are likely to be due to inner retinal neurotransmission.[1]

Electrophysiological studies of humans with diabetes could be used to assess alterations such as dysfunction of ganglion cells and loss of colour and contrast sensitivity; moreover alterations in oscillatory potentials have been shown to predict the onset of proliferative retinopathy better than vascular lesions seen on fundus photographs[2]

Sensitivity of the full-field ERG is limited, precisely because it reflects the activity of the entire retina. Even advanced disease, if confined to small, discrete patches can remain undetected by the full-field ERG[3] .In diabetes, the earliest clinical retinal changes are typically confined to the posterior pole. Therefore, the ability to measure local ERGs in diabetes would improve objective detection of

early functional alterations and assessment of local change over time.

In contrast, the multifocal electroretinography (mfERG) developed by Sutter and Tran[4] enables assessment of up to 103 of distinct retinal areas within approximately 4 minutes per eye. This technique has been applied to the study of retinitis pigmentosa, macular degeneration, glaucoma and diabetes[5]. Studies demonstrated that in some patients with diabetes, mfERG responses (averaged across relatively large areas of retina) were smaller in amplitude and delayed in comparison with those in normal patients[6]. However, they did not determine the extent to which local abnormalities were detected.

The purpose of this study was to identify local retinal abnormalities in diabetic patients with and without non-proliferative diabetic retinopathy (NPDR) using the mfERG.

Material and Method: It was cross sectional study conducted in ERG clinic, in M & J Western Regional institute of ophthalmology, Ahmedabad after obtaining ethical clearance from institutional ethical committee.

The cases were referred to us from ophthalmology. The procedure of examination performed was explained to all the cases and written consent was taken prior to examination. Proper ocular examination was done prior to examination including acuity of vision and corrective lens were given for the test.

The subjects were divided in two groups on the clinical established diagnosis and by fundus examination and compare with 20 normal subjects(32 eyes) with age from 30 to 54 were taken as control who were examined with mfERG under the same condition, using the ISCEV standard [7].Normal subjects were those sent to us for testing malingering or the normal eye in uniocular disease.They had full visual acuity and no history of eye disease or other relevant disorders in the eye taken as control. They were taken as Group A.

In group B were diabetics with normal fundus and no diabetic retinopathy present (no DR).They were 10 subjects with mean age(53± 7.2) years.

In group C were diabetics with changes of diabetic retinopathy (DR) in fundus examination.They were 11 subjects in this group with mean age(57 ±9.1) years.

The stimulus, consisting of 103 hexagons covering a visual field of 50°, was presented on a 9 inch CRT (Cathode ray tube) with a frame rate of 75 Hz at a distance of about 40 cm from the subject's eye. Every 13.3 ms the frame of the monitor changes and each sector has a 50/50 chance of appearing "white" (briefly flashed) or "black" (no flash). The white hexagons were 200 cd/m^2 and the black hexagons the darkest the screen allowed, less than 5 cd/m^2. The area surrounding the array of hexagons was set to 100 cd/m^2 and a central cross was used for fixation. All recordings were performed with the room lights on to help assure a constant state of light adaptation
The duration of the recording session was about 4 minutes, which included 8 recording segments of approximately 30 sec between samples, during which the subjects were not allowed to blink or move.

Both the eyes were dilated with tropicamide (1%) and 2.5% phenylephrine and anesthetized with 0.5% proparacaine. The ERG responses were recorded by means of a bipolar BurianAllen contact electrode which makes use of a large speculum to hold the eyelids apart. A smaller clear corneal contact lens is held against the cornea with a spring assembly. The skin electrode (gold cup electrodes) fixed to the forehead with a conducting pasteserved as a ground electrode. The electroretinogram were amplified (x 50,000 - 100,000) and band pass filtered (10–300 Hz). Recording quality and eye movements were monitored by real-time display and the eye camera, respectively. Contaminated segments were discarded and repeated.The VERIS software 6.1.1 (EDI, San Mateo) developed by Sutter, using a fast m transform algorithm[4] was employed for the calculation and analysis of the 103 local ERG responses from the measured signal. All the data

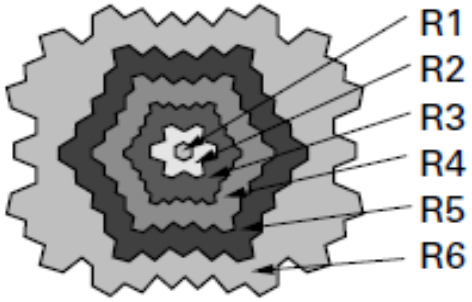
was statistically analysed using student t test and were found to be statistically significant ($p \leq 0.05$)

Result: The visual acuity and fundus finding in the subjects are given in Table 1.For data analysis, the 103 local responses were grouped six concentric rings (R1- R6) centred on the fovea (Fig 1). Response densities and implicit times of the major components N1, P1, N2 in each ring was calculated and analysed.

Table 1: Visual Acuity and fundus finding of the subjects in Group A, B and C

Group	Visual Acuity	Fundus finding
A	6/6 to 6/12	No changes
B	6/6 to 6/12	No changes
C	6/12 to 6/60	Micro aneurysm, retinal haemorrhages. Neovascularisation was found in 1 subject

Fig 1: Concentric rings centering in fovea



Global P1 implicit time was calculated in each eye analysed. As expected in normal subjects the response density decreased from fovea (Ring 1) to eccentricity (Ring 6). In subjects with diabetics - no DR and with DR there was a continuous decrease in response density from the maximum at the fovea towards the periphery. The response density in diabetics without retinopathy was less than normal subjects and more than that of subjects of DR (Fig 2).Student's t test was applied and the difference was found to be statistically significant ($p \leq 0.01$)

Subjects with diabetes without retinopathy showed increased N1 latency compared with those of the control subject. In subjects with diabetic retinopathy the latencies were higher as compared to group B and A (Fig 3)

Fig 2: P1 response density in different concentric ring in Group A (normal), B (no DR) and C (Diabetic Retinopathy)

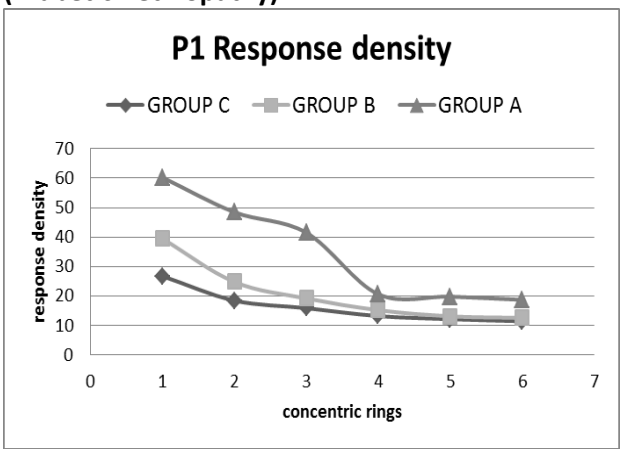


Fig 3: N1 Latency in different concentric ring in group A (normal), B (no DR) and C (Diabetic Retinopathy).

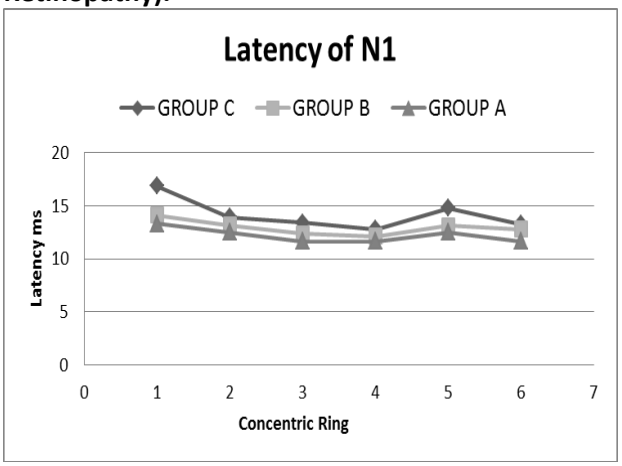
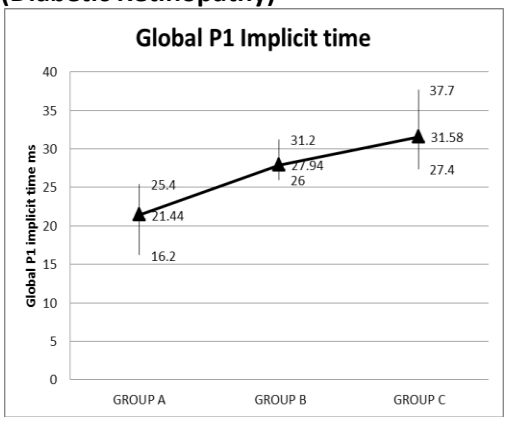


Fig 4: Global P1 Implicit time with the maximum and minimum and the black triangle depicting the mean in each of group A (normal), B (no DR) and C (Diabetic Retinopathy)



P1 implicit time varied throughout different 103 segments of retina. The variation was minimal in normal subjects while the variation of response increased in group B and C. Global P1 implicit time was calculated from the average of the implicit time in all the segments. When compared the mean global P1 implicit time increased from normal to no DR group and DR group (Fig 4)

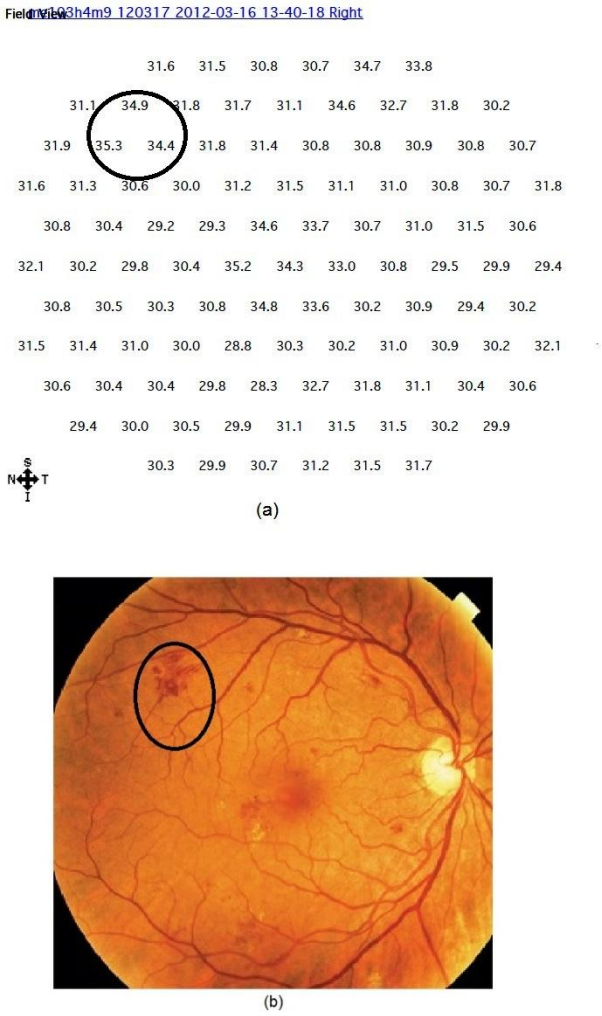
Discussion: We focused on the detection of functional changes in the retina that precede diabetic retinopathy, as the group B in this study showed no pathological changes detectable by standard ophthalmoscopy. The mfERG response revealed a significant amplitude reduction in diabetes.

The results demonstrate that mfERG implicit time analysis is a highly sensitive method of assessment of local retinal function in diabetes. The range of local ERG implicit times observed for the normal eyes in this study was very narrow, consistent with the findings of other mfERG studies[8] .Consequently, local ERG delays as small as 2.5 msec may be regarded as representing significant local retinal dysfunction in diabetic eyes.

In eyes with DR, delays of local responses were greater and were found more throughout the retina than in eyes without retinopathy. Response delays were progressively worse toward the centre of discrete ophthalmoscopic lesions in the retinopathic eye (Fig 5) Smaller, but significant local response delays were found in eyes without retinopathy suggesting that the implicit time analysis revealed subclinical, local retinal dysfunction in these areas. Amplitude variability within diabetic eyes with or without retinopathy was also large (Fig 2). ecently studies[6]reported that implicit times of mfERGs, averaged across the whole retina, were significantly delayed in some diabetic eyes without retinopathy. Whole-field response delays were greater in magnitude and more prevalent among their group of eyes with NPDR.

In the above figure Superior nasal part shows the retinal haemorrhages in fundus photograph marked by black circle and increase in P1 Implicit time as compared to neiHbA1couring segments is found in similar segment of P1 implicit time array.

Fig 5: (a) P1 implicit time in right eye of a patient of Non-progressive DR (b) Fundus photograph of the right eye of same patient.



Timing changes appear to represent neural response or conduction delays secondary to compromised local metabolism and/or blood flow. Even the early features of the diabetic responses (first trough and peak, or a- and b-wave analogues, respectively) appeared to be delayed. This suggests that the generators of early response components may be functionally compromised within these retinal regions. The initial negative and positive voltage deflections of the mfERG have been shown to behave much like the components of the photopic, full-field flash ERG and are likely to be generated by the same retinal elements [9.] Based on this parallel, it is possible that some of the

response timing changes observed here represent compromised function in the outer retina (cone photoreceptors) and/or middle retina (cone bipolar cells, Muller cells) secondary to diabetes.

It is possible that such early local ERG changes, found in the absence of retinal vascular findings, are caused by early diabetic choroidal lesions[10]. Retinal hypoxia is thought to be a major stimulus leading to increased expression of vascular endothelial growth factor and vascular permeability factor (VEGF/VPF)[11], although increased glucose concentration alone may be sufficiently damaging[12] In turn, increased expression of VEGF/VPF is likely to be a critical factor in the development of even the earliest retinal vascular lesions in NPDR[13]. In fact, local breakdown of the blood–retinal barrier has been associated with increased immunoreactivity for VEGF/VPF in the early stages of experimental diabetic retinopathy, as well as in diabetic human eyes in patients in whom fellow eyes had no evidence of retinopathy[14]

Taken together, these results suggest that the mfERG may serve to monitor local metabolic conditions that lead to (or are related to) the development of diabetic retinal vascular lesions such as breakdown of the blood–retinal barrier. Use of the mfERG may also improve objective follow-up of treatment interventions.

Conclusion: The presence of significant local response delays in eyes without clinically evident retinopathy suggests that such mfERG changes may provide a very early indicator of local retinal dysfunction in diabetes. Observing these subjects longitudinally will help determine whether abnormal mfERG responses (timing delays, in particular) predict development and/or progression of retinopathy in discrete retinal locations.

In summary, we believe that the results presented here demonstrate that implicit time delays of multifocal ERGs reveal abnormal local retinal function in diabetes corresponding to local, discrete retinopathic lesions. The mfERG is easily obtained in a clinical setting, and provides a very sensitive, objective assessment of local retinal health in diabetes.

References:

1. María Miranda , María Victoria Sánchez - Villarejo, Raquel Álvarez-Nölting, Concha Vilela and Francisco Javier Romero. Electroretinogram Alterations in Diabetes?, In Electroretinograms, Dr. Gregor Belusic (Ed.),2011:157-172
2. Fortune B, Bearse MA, Cioffi GA, et al. Selective loss of an oscillatory component from temporal retinal multifocal ERG responses. Invest Ophthalmol Vis Sci 2002; 43:2638-47.
3. Birch DG. Focal electroretinography. In: Heckenlively JR, Arden GB, eds. Principles and Practice of Clinical Electrophysiology of Vision 2nd edition . St. Louis: Mosby; 2006:319-341.
4. Sutter EE. the fast m-transform: a fast computation of cross correlations with binary m-sequences. Soc Ind Appl Math 1991;20: 686–694
5. Donald CH , Jeffrey GO, Candice SC, The Multifocal Electroretinography. J Neuro-Ophthalmol, Vol. 23, No. 3, 2003:225-235
6. Palmowski AM, Sutter EE, Bearse MA, Fung W. Mapping of retinal function in diabetic retinopathy using the multifocal electroretinography. Invest Ophthalmol Vis Sci. 1997;38:2586–2596.
7. Donald CH, Michael B (for the International Society For Clinical Electrophysiology of Vision). Standard for Clinical electroretinography (2011 update). Doc Ophthalmol (2012) 124:1–13
8. Verdon WA, Haegerstrom–Portnoy G. Topography of the multifocal electroretinography. Doc Ophthalmol. 1998;95:73–90.
9. Hood DC, Seiple W, Holopigian K, Greenstein V. A comparison of the components of the multifocal and full-field ERGs. Vis Neurosci. 1997;14:533–544.
10. Cao J, McLeod DS, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol. 1998;116:589–597.
11. Aiello LP, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular

endothelial growth factor in retinal cells. Arch Ophthalmol. 1995;113:1538–1544.
12. Natarajan R, Bai W, Lanting L, Gonzales N, Nadler J. Effects of high glucose on vascular endothelial growth factor expression in vascular smooth muscle cells. Am J Physiol. 1997;273:H2224–H2231.
13. Mathews MK, Merges C, McLeod DS, Lutty GA. Vascular endothelial growth factor and vascular permeability changes in human diabetic retinopathy. Invest Ophthalmol Vis Sci. 1997;38:2729– 2741.
14. Murata T, Nakagawa K, Khalil A, Ishibashi T, Inomata H, Sueishi K. The relation between expression of vascular endothelial growth factor and breakdown of the blood-retinal barrier in diabetic rat retinas. Lab Invest. 1996;74:819–825

Source Of Financial Support-Nil
Conflict Of Interest-None