Comparison of Photopic Negative Response of Electroretinogram in Non-Diabetic and Diabetic Indian Subjects

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ABSTRACT

Purpose: To determine how photopic negative response (PhNR) of elctroretinogram (ERG) is affected in diabetes subjects with and without retinopathy and to compare it with the controls in Indian subjects.

Methods: Fifty-nine subjects were divided into control, diabetes with no retinopathy, non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR) groups. All subjects photopic ERG parameters were measured using short duration (4 milli seconds) red flashes (1.7 cd..m^{-2}) on blue background (8cd. m^{-2}). Photopic ERG parameters, a-wave amplitude, a-wave implicit time, b-wave amplitude, b-wave implicit time and PhNR amplitudes were measured in all the subjects.

Results: 25 eyes of 14 subjects with diabetes, 37 eyes of 30 subjects with various stages of diabetic retinopathy and 24 eyes of 15 age matched control subjects were studied. There is a significant reduction ($p < 0.05$) in PhNR amplitude along with other ERG parameters such as a-wave amplitude, a-wave implicit time, b-wave amplitude, b-wave implicit time in diabetic retinopathy subjects when compared to control and diabetes subjects but there is no significant difference ($p > 0.05$) in photopic ERG parameters between controls and diabetic subjects and also between NPDR and PDR subjects.

Conclusion: PhNR amplitude along with the other photpic ERG parameters is useful for the evaluation of inner retinal function in cases of diabetic retinopathy when compared to early stages of diabetes.

Key words: Photopic Negative response, electroretinogram, diabetes, non proliferative, proliferative, retinopathy.

INTRODUCTION

Diabetes is a group of metabolic diseases characterised by hyperglycaemia and glucose intolerance.[1] According to World Health Organisation (WHO) report in 2014, it is estimated that 422 million people were suffering with diabetes and its prevalence is rapidly raising in low and middle income countries. Diabetes often affects different organs of the body including the microvasculature of the retina leading to diabetic retinopathy (DR) which is one of the leading causes of visual impairment globally.[2] There are approximately 90 million people living with DR.[3] According to Center for Disease Control and Prevention: national diabetes fact sheet (2007) in the US, DR is responsible for 12,000 to 24,000 new cases of blindness annually raising considerable public health concern.

QConsidering the nature of the problem, it is important to detect the DR early that can lead to better intervention strategies to avoid blindness. Recently, there is growing interest in studying the inner retinal function of diabetes subjects especially function of retinal ganglion cells as they are more susceptible to damage.[4,5] Studies related to structural changes in diabetes subjects using optical coherence tomography clearly showed that there is significant reduction in RGCL and NFL layer thickness in comparison to normal subjects.[6,7,8] In addition to structural changes functional deficits in inner retina has also been shown in early stages of DR. Various studies have used flash ERG parameters to study inner retinal function in diabetic subjects and found that ERG parameters affected in different stage of diabetic retinal disease. In

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diabetic subject's significant reduction in the amplitude and increased implicit time of the oscillatory potentials (OPs) were noticed. In earlier stages of DR, the changes in ERG parameters other than oscillatory potentials were not significant. The decrease in OPs was correlated to severity of DR. Patients with severe NPDR showed significantly reduced and sometimes absent OPs.9].[10,11] Under photopic (light adapted) conditions a negative wave following b-wave in Flash Electroretinogram is described as photopic negative response (PhNR) and was first identified by Vishwanathan et al. in 1999 and found to be originating mainly from ganglion cells.[12] Studies have shown that the amplitude of the PhNR is reduced in patients with primary open-angle glaucoma (POAG)[13-16] optic nerve atrophy, autosomal dominant optic atrophy with OPA1 mutations,[17, 18] central retinal artery occlusion (CRAO) and branch retinal vein occlusion (BRVO).[19] The decrease in amplitude of PhNR was progressively correlated with the progression of DR.[10, 20] Though there are many studies eliciting the PhNR responses in normal and diabetic retinopathy subjects, as per our knowledge, there were hardly few studies that compared the changes in PhNR response in diabetic patients with and without retinopathy. As per our knowledge PhNR response is not reported from diabetic Indian subjects in any of the prior studies. Considering the research gap, current study aimed at comparing the photopic negative response (PhNR) and other photopic ERG parameters in control and diabetes patients with and without retinopathy in Indian subjects.

QVarious factors such as stimulus strength and colour, background intensity, stimulus duration and age seem to influence the PhNR amplitude and implicit times.[16, 19-21] Brief and long duration flashes are generally used for eliciting PhNR. Brief flashes (< 5ms) are convenient and commonly used in clinical settings. Different studies used different stimulus strength for electing PhNR. Recently International Society for Clinical Electrophysiology of Vision (ISCEV) have proposed optimal standards for recording PhNR response using extended protocol.[22] Though our study was conducted before ISCEV proposed standards for PhNR, our stimulus protocol and recording parameters are well aligned with ISCEV standards.

METHODS AND MATERIALS

Subjects: Total of 59 subjects (15 controls, 44 subjects with diabetes) aged 40-80 years presented to outpatient department of L.V Prasad Eye institute were considered for the study. This study was approved by the Institution Ethics committee of the L.V. Prasad Eye Hospital and Institute, Hyderabad, India and the study was conducted following the tenets of the Declarations of Helsinki medical research involving human subjects. All the study population was informed about the study and prior written and oral consent was taken from all the subjects. The eyes of the subjects who is having cataract or any media opacity were not included in the study. Patients already underwent prior cataract surgery without any complications were included in the study

Total 59 subjects were divided into four groups. The groups are controls, diabetic subjects without retinopathy, subjects with non-proliferative diabetic retinopathy (NPDR) and subjects with proliferative diabetic retinopathy (PDR). NPDR and PDR subjects are classified based on the international clinical diabetic retinopathy disease severity scale by an ophthalmologist specialised in retina. Controls were defined as the subjects who are having BCVA of 20/20 with normal anterior and posterior segment of the eye and without any systemic disorders.

All subjects underwent comprehensive eye examination and patients with best corrected visual acuity less than 20/200, having any systemic disease other than diabetes, having any ocular pathology and also patients who underwent laser treatment of the retina for any reason were excluded from the study.

PhNR recordings & Electrode placement: All subject pupils were maximally dilated (minimum of 8mm) with Itrop plus (0.8% Tropicamide and 5% Phenylephrine, Cipla Pharmaceuticals). LVPEI ZARI electrode (low mass silver impregnated microfiber) is used as an active electrode (positive electrode) and placed in the lower fornix just touching the lower limbus with a clip and then secured at the inner (nasal) and outer canthi with tape. Silver-Silver chloride cup electrodes were filled with conductive gel. Two electrodes were taped over the ipsilateral outer canthus of each eye which acted as reference electrodes and other electrode is placed on the forehead acted as ground electrode. Before placing the electrodes, the skin under the reference and ground electrodes was cleaned with Nuprep gel. Then the subjects are instructed to place their chin on chin rest supported by forehead rest.

The PhNR was recorded using the Metro vision instrument (Mon pack ONE) using red flashes (intensity1.7cd s/ m^2) on blue background (intensity 8cd s/ m^2) by prior adapting the subjects to blue background for 5 minutes. Flashes were produced by light-emitting diodes with a duration of 4 ms. Electric signals were amplified 1000 times and were digitalised using analog to digital converter. Signal cut-off frequency was set between 0.3 Hz to 300Hz. Average of 100 responses was used to analyse the photopic ERG parameters.

The amplitude of a-wave is measured from the baseline to the negative trough of the a-wave, and the amplitude of the

b-wave is measured from the trough of the a-wave to the following peak of the b-wave. The implicit time a-wave is measured from the time onset of flash to the trough of the a-wave and the b-wave implicit time is measured from the time onset of flash to the peak of the b-wave. To standardize PhNR responses in all subjects The amplitude of PhNR was measured from peak of the b- wave to trough that seen between 68-80 ms.

Statistical Analysis: The PhNR and other ERG parameters were entered into excel sheet data and data was transferred to SPPS 17.0. One-way ANOVA was performed to see the difference in all ERG parameters between the four groups and statistical significance was set at $p \leq 0.05$.

RESULTS

Out of 59 subjects control group consisted of 24 eyes of 15 subjects with mean age of 49.6 ±7.9 years. The diabetic group without retinopathy consisted of 25eyes of 14 subjects with mean age of 52.5 ± 7.0 years. NPDR group consisted of 22 eyes of 15 subjects with mean age 54.1± 7.0 years and PDR group consisted of 15 eyes of 15 subjects with mean age 55.0 ± 8.50years. Mean amplitudes and implicit times of all ERG parameters were presented in Table 1.

In the control group, the mean amplitude of a-wave, b-wave and PhNR were was 16.5 ± 3.0µV, 53.2 ± 7.8µV and 24.0 ± 5.9 µV respectively while mean implicit times were 18.5 ± 1.00 ms and 33.2 ± 1.2 ms respectively.

In the diabetic group, the mean amplitude of a-wave, b-wave and PhNR were was 17.7 ± 2.4 µV, 52.4 ± 5.2µV and 20.5 ± 5.4 µV respectively while mean implicit times were 18.9± 1.0 ms and 33.2 ± 1.2 ms respectively.

In the NPDR group, the mean amplitude of a-wave, b-wave and PhNR were was 12.6 ± 2.7 µV, 44.4 ± 7.1µV and 11.5 ± 5.2 µV respectively while mean implicit times were 20.5 ± 1.3 ms and 37.5 ± 2.2 ms respectively.

In the PDR group, the mean amplitude of a-wave, b-wave and PhNR were was 12.4 ± 4.4 µV, 41.4 ± 6.5µV and 6.5 ± 4 µV respectively while mean implicit times were 21.4 ± 1.6 ms and 39.4 ± 3.0 ms respectively.

PhNR response of four individuals were presented in Figure 1 and it clearly shows decreased b-wave and PhNR amplitude in NPDR and PDR subjects in comparison to normal and diabetic subject. Control and Diabetic and NPDR subject shows almost similar waveform but it is significantly varying in PDR subject suggests that inner retinal functional damage is severe in PDR.

Mean amplitudes and implicit times of all ERG parameters i.e a-wave amplitude, b-wave amplitude and PhNR amplitude were presented in Figure 2a and similarly a-wave implicit time and b-wave implicit time of four groups were presented in figure 2b). There is a clear trend in the amplitude data that there is clear decrease in mean a-wave amplitude and mean b-wave amplitude and mean PhNR amplitude in diabetic retinopathy groups in comparison to control and diabetes with no retinopathy groups. Similarly b-wave mean implicit time is significantly increased in diabetic retinopathy groups in comparison to control and diabetes with no retinopathy groups.

Analysis of variance (ANOVA) was performed to see is there any differences exist within the groups and also between the groups. ANOVA shows that there is a significant difference in amplitudes of PhNR, a-wave and b-wave between the four groups ($p<0.001$) (Table 1). Similarly, the implicit times of the a-waves, b-waves showed significant differences ($p<0.001$) between the four groups. On further analysis with Turkey HSD post-hoc test there was significant differences ($p<0.001$) were noted in mean a-wave amplitude, mean a-wave implicit time, mean b-wave amplitude, mean b- wave implicit time and mean PhNR amplitude between control and NPDR group, control and PDR group, diabetes and NPDR group and also diabetes and PDR group.

Table 1: *Mean values a-wave amplitude, a-wave implicit time, b-wave amplitude, b-wave implicit time and PhNR amplitude in four groups of subjects.*

	Control	Diabetes	NPDR	PDR	p-value
Patients (eyes)	15 (24)	15 (25)	15(22)	15(11)	
Age (yr)	49.6 ±7.9	52.5 ± 7.0	54.1± 7.0	55.0 ± 8.50	0.103
a-wave					
amplitude (µV)	16.5 ± 3.0	17.7 ± 2.4	12.6 ± 2.7	12.4 ± 4.4	<0.001
Implicit time (ms)	18.5 ± 1.0	18.9 ± 1.0	20.5 ±1.3	21.4 ± 1.6	<0.001
b-wave					
amplitude (µV)	53.2 ± 7.8	52.4 ± 5.2	44.4 ± 7.1	41.4 ± 6.5	<0.001
Implicit time (ms)	33.2 ± 1.2	33.0 ± 1.5	37.5 ± 2.2	39.4 ± 3.0	<0.001
PhNR					
Amplitude	24.0 ± 5.9	20.5 ± 5.4	11.5 ± 5.2	6.5 ± 4.0	<0.001

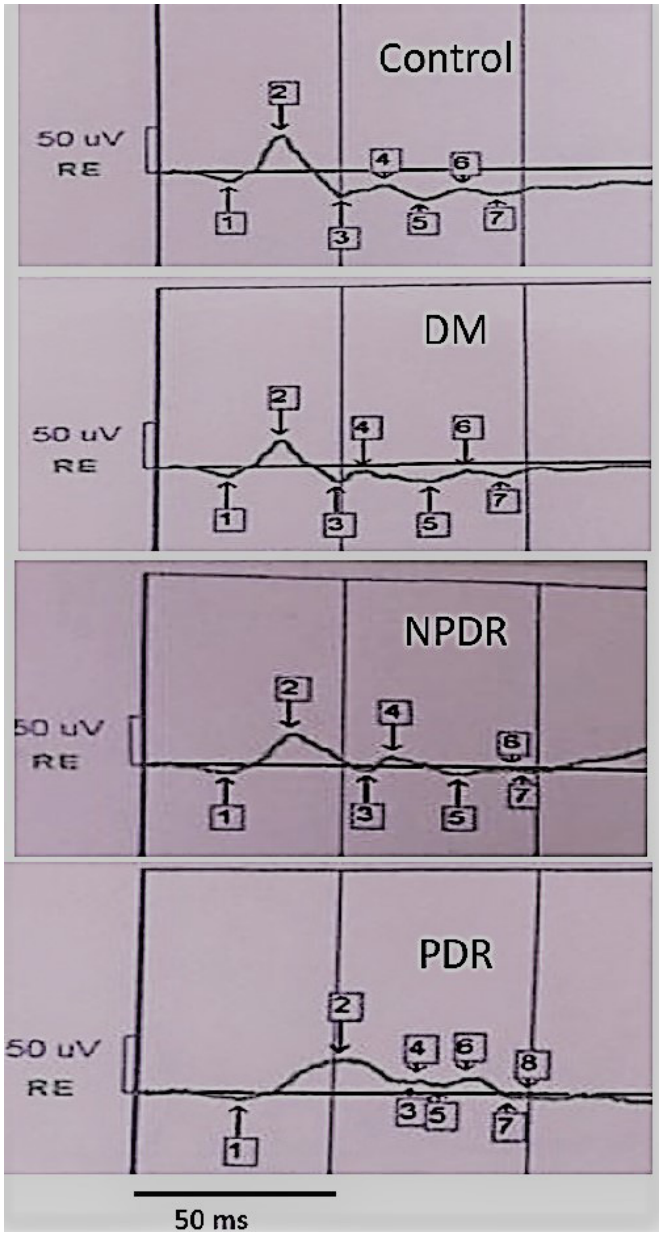

Figure 1:

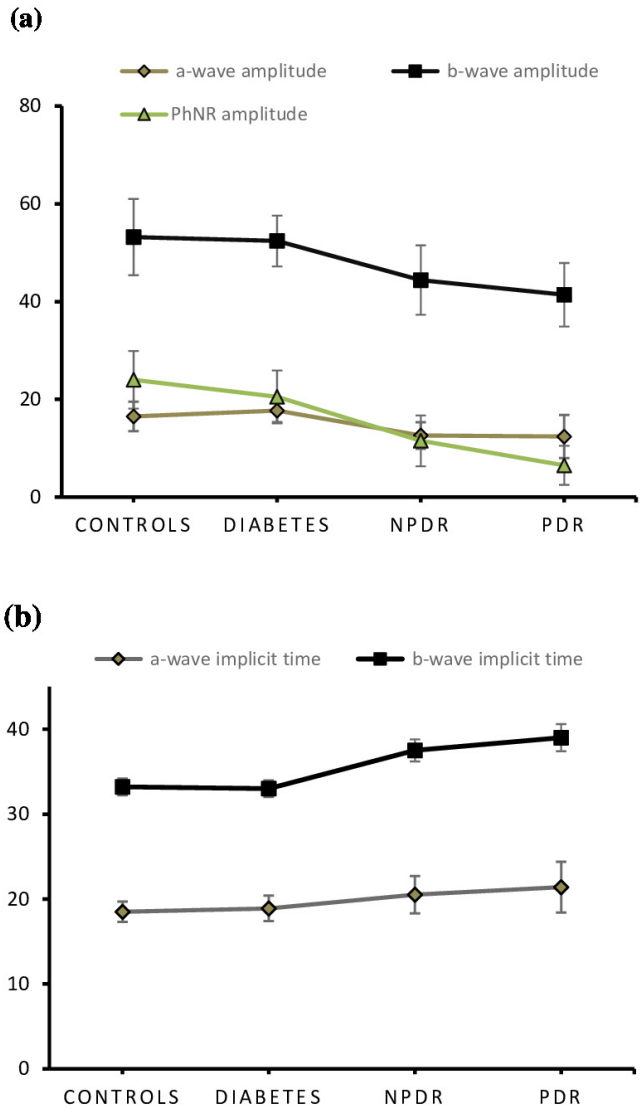

Figure 2:

DISCUSSION

ERG is an objective and non-invasive technique that helps to measure the neural function of the retina. Traditionally, photopic ERG parameters and PhNR were attributed to the cone bipolar and ganglion cell response respectively.[12, 23-25] Current study shows that the PhNR amplitude was found to be significantly decreased in diabetic retinopathy subjects when compared to control and diabetes subjects. Along with PhNR amplitude, a-wave amplitude, b-wave amplitudes were also significantly decreased and a-wave implicit time and b-wave implicit time is significantly increased in NPDR and PDR subjects when compared to controls and diabetes subjects. If this is the case, results from the current study suggest that cone bipolar pathway and retinal ganglion cells are affected in NPDR and PDR subjects and not noticeable in diabetic subjects. The findings of the current study also suggest that retinal neural function is not affected in diabetic subjects until they develop vascular abnormalities.

PhNR can be a sensitive indicator even if the patient has early diabetic retinopathy and it is strongly supported by Chen et al study that reported there is a decrease in PhNR response between controls and early NPDR subjects.10]. However current study not showed any significant difference in PhNR amplitude between the NPDR and PDR group due to two possible reasons; **a)** small sample size in PDR group compare to NPDR group and **b)** most of the patients in NPDR group were with moderate to severe grade, so amount of damage to inner neural retinal function of NPDR group may be closely similar to the PDR group in this study. Adding mild NPDR subjects in this study would have influenced the outcome and would have

been shown the significant difference in ERG parameters between NPDR and PDR groups.

There are very few studies that compared PhNR in normal, diabetic and diabetic retinopathy subjects.[10, 27] As per our knowledge this is the first and only study from India reporting the PhNR in diabetic subjects comparing normal and diabetic retinopathy subjects.

The results of these studies shows that there is no significant difference in PhNR amplitude between control and diabetic group but there is significant difference in PhNR amplitude between control and diabetic retinopathy group. Park et al (2017) showed there is decrease in mean PhNR amplitude of 8% between control and non diabetic retinopathy group and 35% between control and diabetic retinopathy group.[27] Observation from current study shows that there is nearly 15% reduction in PhNR amplitudes between controls and patients with diabetes without retinopathy and 55% reduction between controls and NPDR group and 75% between controls and PDR group. Current study results are in accordance with previously reported data. furthermore current data shows that PDR patients have significant reduction in PhNR amplitude when compared to NPDR group and suggest that PhNR response is a better tool in future to see the nerural damage within the diabetic retinopathy groups. .

Study done by Kim et al has showed that there was delay in PhNR implicit time in diabetic retinopathy subjects in comparison to control group.[15] One of the limitations of this study is that PhNR implicit time was not analysed but general observation from the waveform data is that implicit times were delayed in NPDR and PDR groups in comparison to control and diabetic group. Though the current study shows some insights about understanding of photopic flash ERG parameters in normal, diabetic and diabetic retinopathy subjects, it has only few subjects in the mild NPDR group. Future studies using larger samples are needed to confirm the findings of current study.

CONCLUSION

Current study shows that the PhNR is very useful tool for evaluating inner retina function in patients with diabetic retinopathy but may not be sensitive indicator in diabetic subjects without retinopathy in Indian population.

Conflict of Interest statement: Authors listed in the current study has no conflict of interest with any organization or industry.

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