Original Article

Comparison of broadband and monochromatic photopic negative response in eyes of patients with diabetes with no diabetic retinopathy and different stages of diabetic retinopathy

Aniruddha Banerjee[1,2], Karthika Pandurangan[1], Amose Joe[1,2], Ramya Sachidanandam[1,2], Parveen Sen[2]

Purpose: To evaluate the change in broadband (W/W), red on blue (R/B), and blue on yellow (B/Y) photopic negative response (PhNR) in patients with diabetes mellitus with no diabetic retinopathy (no DR) and different stages of DR and compare it with age-matched controls. This study was performed to provide a single PhNR protocol that can be used for early diagnosis of DR. **Methods:** It was a cross-sectional case-control study done in a hospital setup. Patients with diabetes with no DR and different stages of DR with no other associated ocular pathologies were included. Age-matched controls with no retinal pathologies were also included for comparison. All subjects underwent detailed ophthalmic examination and W/W, R/B, and B/Y electroretinography. Fifty control eyes and 52 treatment naïve eyes of 52 patients with diabetes [no DR = 11, mild nonproliferative diabetic retinopathy (NPDR) =11, moderate NPDR = 10, severe NPDR = 9, and proliferative DR = 11] were included in the study. **Results:** On comparing the ERG responses in patients with diabetes and age-matched controls, a significant reduction ($P < 0.05$) was noted in the amplitudes of a-wave (39.78 ± 11.34 μV vs. 67.28 ± 12.88 μV), b-wave (116.25 ± 45.25 vs. 134.39 ± 28.78 μV), W/W PhNR (33.86 ± 17.33 vs. 67.18 ± 15.99 μV), R/B PhNR (28.77 ± 15.85 vs. 53.48 ± 14.15 μV), and B/Y PhNR (55.04 ± 32.63 vs. 104.79 ± 24.37 μV). *Post hoc* analysis revealed that all the eyes in the diabetic group, including those with no DR, had a significantly reduced PhNR amplitude ($P < 0.05$) when compared with controls. PhNR was found to reduce in amplitude with increasing severity of DR ($P < 0.05$), with more significance in B/Y. Receiver operating characteristic showed highest area under the curve in B/Y PhNR (94%, $P < 0.001$), with maximum sensitivity and specificity of 88% and 87%, respectively. **Conclusion:** Changes in the amplitude and implicit time of ERG can reflect the severity of DR. PhNR amplitudes, especially B/Y PhNR, appear to be significantly reduced even in eyes with no DR.

Access this article online

Website: www.ijo.in

DOI: 10.4103/ijo.IJO_988_21

Quick Response Code:

Key words: Diabetic retinopathy, electroretinography, retinal ganglion cell

Diabetic retinopathy (DR), till recently, has been described as a chronic microvascular complication seen in the retina due to systemic diabetes.[1] However, the theory of retinal neurodegeneration in the early stages of the disease has recently gained importance. Many factors have been linked to the neuroretinal damage in diabetes, including neural apoptosis of retinal cells (ganglion, amacrine, and muller cells), increased expression of the glial fibrillary acidic protein in Muller cells, reduction in neuroprotective factors, and glutamate excitotoxicity.[2,3] Studies report a control in the severity of DR associated with good glycemic control.[4]

Photopic negative response (PhNR) is a slow, negative-going response after b-wave in full-field electroretinogram (ERG). It was first observed by Viswanathan *et al*.[5] and examined on experimental monkeys by injecting tetrodotoxin. The PhNR amplitude has been found to be reduced in human glaucoma subjects[5-7] and has been reported to be a sensitive biomarker of retinal ischemia and DR.[8-10] A study by Kim *et al*.[8] reported a reduction in amplitude and a delay in implicit time of white-on-white (W/W) broadband PhNR in eyes with moderate to severe nonproliferative diabetic retinopathy (NPDR) even when the a-wave and b-wave were unaffected. Reduction in amplitude and delay in implicit time of PhNR was also found in patients with diabetes but no clinical DR (no DR) compared to controls using a red flash over a blue background.[9] Blue flashes over amber background ERG obtained from subjects with adolescent type 1 diabetes also showed a reduced PhNR amplitude with a delayed implicit time.[10] The role of PhNR in early detection of diabetic retinopathy has not been studied extensively, especially using monochromatic stimuli. In the present study, we aimed to evaluate different chromatic stimuli to elicit PhNR and determine the effect of severity of DR on ERG parameters. W/W stimulus was used to obtain mass response from all the cone photoreceptors,[11] and different

Departments of [1]Optometry and [2]Vitreo-Retinal Services, Medical Research Foundation, Chennai, Tamil Nadu, India

Correspondence to: Dr. Parveen Sen, Department of Retina, Shri Bhagwan Mahavir Vitreoretinal Services, Sankara Nethralaya, Medical Research Foundation, 18 College Road, Nungambakkam, Chennai – 600 006, Tamil Nadu, India. E-mail: parveensen@gmail.com

Received: 29-Apr-2021 Revision: 10-Jun-2021
Accepted: 27-Aug-2021 Published: 29-Oct-2021

Cite this article as: Banerjee A, Pandurangan K, Joe A, Sachidanandam R, Sen P. Comparison of broadband and monochromatic photopic negative response in eyes of patients with diabetes with no diabetic retinopathy and different stages of diabetic retinopathy. Indian J Ophthalmol 2021;69:3241-8.

monochromatic stimuli were used to selectively stimulate different cones and ganglion cell types present in the retina.[12]

Methods

The study was performed according to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained prior to examination from all participants after an explanation of the nature and possible consequences of the study. This was a cross-sectional, observational, case-control study. Fifty-two eyes of 52 subjects with diabetes and 50 age-matched healthy participants (50 eyes, aged 30–70 years) were recruited as cases and controls, respectively. Healthy subjects with no ocular pathology, no history of ocular and traumatic brain injury, ocular surgery, steroid intake, or lazy eye, and having visual acuity better than or equal to 20/25 were included in the control group. Eyes with myopia and hypermetropia more than ±5.00 D (spherical equivalent) in any of the groups were excluded from the study. Subjects with a history of diabetes were included in the DR group. Treatment naïve eyes were included and classified into no DR, mild NPDR, moderate NPDR, severe NPDR, and proliferative DR (PDR). The classification of DR was based on funduscopic findings and was in accordance with the International Clinical Diabetic Retinopathy Disease Severity Scale.[13] Eyes with diabetic macular edema were also excluded from the study.

After 10 min of background light adaptation, the photopic ERG was recorded simultaneously in both the eyes after complete pupillary dilatation with 1% tropicamide for all the participants using VERIS Ganzfeld (Visual-Evoked Response Imaging System, version 6.4.2; Electro-Diagnostic Imaging Inc., Redwood City, CA). Burian–Allen contact lens electrodes were used as active electrodes and were placed on both the eyes after instilling a drop of topical anesthesia. Lubricating eye drops were instilled over the concave side of the polymethyl methacrylate (PMMA) lens to prevent the cornea from dryness. A gold cup electrode placed on the earlobe after application of electroconductive paste was considered the ground electrode. Electrode placement was as per the guidelines of the International Standard of Clinical Electrophysiology and Vision science.[14] The test procedure was performed for each color stimuli. The second negative lowest trough, after the b-wave and i-wave measured from the baseline, was considered as the PhNR wave.

In the control group, only right eyes were included, and in the diabetic group, one eye of each individual (worse eye, based on the DR severity scale[13]) was included for analysis. The median time duration of total procedure per patient was 2.4 h, which included clinical workup, dilation, fundus photo, optical coherence tomography (OCT), and three ERG protocols.

To begin with, W/W PhNR was done with a flash strength of 3.50 cd.s/m^2 over 10 cd/m^2 after 10 min of background adaptation. Following W/W, R/B PhNR was done using a 3.50-cd.s/m^2 red flash (peak wavelength: 635 nm) and was projected on a 10-cd/m^2 blue background (peak wavelength: 450 nm) after 10 min of background adaptation. Following this, B/Y PhNR was performed using a blue (peak wavelength: 448 nm) stimulus of 1.00-cd.s/m^2 flash strength and was projected over a 10-cd/m^2 yellow (peak wavelength: 592 nm) after 10 min of background adaptation. The order and adaptation protocol were the same for all patients in both groups. Each patient was presented 50 flashes for all protocols before averaging 10 well-defined waveforms for further analysis. Duration of all flashes was kept constant (4 ms). Responses were amplified at 5K gain, and bandwidth filter frequency was set at 0.3–1000 Hz. The accuracy of color wavelengths was confirmed by using a photometer (PR655, SpectraScan, Spectroradiometer, Photoresearch, Inc). Selection of specific flash strengths and background intensities from all the three protocols were taken from our earlier published paper[15] where we selected a specific flash strength and background based on the response with the most well-defined peak before the saturation occurs.

All subjects of the diabetic group underwent Fundus photography (FF 450 Plus with Visupac, Zeiss, USA) and optical coherence tomography (Cirrus™ HD-OCT 500, Carl Zeiss Meditec, Dublin, CA) before ERG test (minimum 30 min prior) for documentation and classification of DR.

Statistical analysis

Mean age was compared between the two groups by using independent sample *t*-test. Comparison of W/W, R/B, and B/Y responses between controls (n = 50) and diabetic group (n = 52) was done using independent sample *t*-test. Comparison among different severities of the diabetic group was done using ANOVA, and *post hoc* analysis was done with a Bonferroni test with a conservative *P* value of 0.008. Independent sample *t*-test was done between controls and stages of retinopathy ($P < 0.05$). A receiver operating characteristic (ROC) curve analysis was done between W/W, R/B, and B/Y PhNR in controls and in the DR group. Data entry was done in Microsoft Excel, version 2010, and statistical analysis was performed using IBM® Statistical Package for the Social Sciences (SPSS), version 20.

Results

All the subjects in the control group (50 eyes) had a visual acuity of 20/20, with their refractive error lying between ±5.00 D (spherical equivalent). A total of 50 control eyes and 52 eyes in the diabetic group were included and analyzed.

The mean age of controls was 50.06 ± 9.43 years (males = 21; females = 29), while the mean age of the 52 subjects in the diabetic group (males = 32; females = 20) was 56.19 ± 8.08 years. The mean age was not significantly different ($P = 0.05$) between the two groups.

A Flash strength of 3.50 cd.s/m^2 for W/W and R/B and of 1.00 cd.s/m^2 for B/Y ERG were used for comparison between controls (n = 50) and diabetic group (n = 52). The amplitudes of a-wave, b-wave, and PhNR in the diabetic group as compared to controls were significantly reduced ($P = < 0.05$) [Tables 1a-1c]. The delay in mean implicit time between the diabetic group and control eyes in W/W PhNR (7.99 ms), R/B PhNR (9.18 ms), and B/Y PhNR (2.90 ms) was less compared to the reduction in amplitudes [Tables 1a-1c].

The 52 eyes from the diabetic group were subdivided as no DR (n = 11), mild NPDR (n = 11), moderate NPDR (n = 10), severe NPDR (n = 9), and PDR (n = 11). ERG in these eyes, when compared to controls, showed a statistically significant decrease in the amplitudes and delay in implicit time ($P < 0.05$) [Tables 2a-2c] in all three color stimuli except

Table 1a: Amplitudes and implicit times of broadband ERG in controls and diabetic eyes

	a-wave		b-wave		PhNR	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	67.28±12.88	16.72±1.01	134.39±28.78	38.07±1.70	67.18±15.99	72.56±7.61
Diabetic (*n*=52)	39.78±11.34	19.57±1.64	116.25±45.25	37.91±4.20	33.86±17.33	80.55±9.24
Mean difference (CI)	27.50 (22.74-32.26)	−2.85 (−4.38-−1.31)	17.86 (2.90-32.83)	−1.84 (−3.11-−0.57)	33.31 (26.75-39.87)	−7.99 (−11.32-−4.67)
P	<0.0001*	<0.0001*	0.02*	0.005*	<0.0001*	<0.0001*

*Statistically significant, Independent sample *t*-test was performed (*P*<0.05), CI=Confidence interval, PhNR: Photopic negative response

Table 1b: Amplitudes and implicit times of red on blue ERG in controls and diabetic eyes

	a-wave		b-wave		PhNR	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	50.45±10.02	16.59±0.95	132.02±23.97	35.16±2.05	53.48±14.15	72.99±5.43
Diabetic (*n*=52)	36.81±10.24	18.89±1.70	112.09±35.57	40.04±6.28	28.77±15.85	82.18±8.98
Mean difference (CI)	13.63 (9.65-17.62)	−2.29 (−2.83-−1.74)	19.92 (7.96-31.88)	−4.88 (−6.73-−3.03)	24.71 (18.80-30.62)	−9.18 (−12.12-−6.25)
P	<0.0001*	<0.0001*	0.001*	<0.0001*	<0.0001*	<0.0001*

*Statistically significant, Independent sample *t*-test was performed (*P*=<0.05), CI=Confidence interval, PhNR: Photopic negative response

Table 1c: Amplitudes and implicit times of blue on yellow ERG in controls and diabetic eyes

	a-wave		b-wave		PhNR	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	103.94±22.18	17.38±1.55	195.46±52.22	36.61±9.30	107.68±25.37	101.22±19.46
Diabetic (*n*=52)	46.37±20.73	21.19±3.06	112.72±41.87	39.98±5.23	55.04±32.63	103.32±20.93
Mean difference (CI)	57.57 (49.13-66.00)	−3.81 (−4.77-−2.85)	82.74 (64.18-101.30)	−3.36 (−6.31-−0.41)	52.64 (41.12-64.15)	2.09 (−10.04-−5.85)
P	<0.0001*	<0.0001*	<0.0001*	0.02*	<0.0001*	0.6

*Statistically significant, Independent sample *t*-test was performed (*P*=<0.05), CI=Confidence interval, PhNR: Photopic negative response

W/W b-wave implicit time ($P = 0.51$), B/Y b-wave implicit time ($P = 0.14$), and B/Y PhNR implicit time ($P = 0.63$).

Bonferroni *post hoc* analysis of ERG parameters with different stages of DR is given in Tables 2a, 2b, and 2c. The comparison between the control and diabetic groups (control vs. no DR, control vs. mild NPDR, control vs. moderate NPDR, control vs. severe NPDR, and control vs. PDR) showed a reduction in mean amplitude in all PhNR parameters as the severity of DR increased [Table 3]. The a-wave and b-wave amplitudes were also reduced in all stages of diabetic retinopathy but the decrease was less significant as compared to the decrease in all PhNR amplitudes (W/W, R/B, and B/Y). All three PhNR amplitudes were significantly low even in the no DR and mild NPDR groups ($P < 0.05$) as compared to controls. In the control and no DR groups, the mean amplitude reduction in B/Y PhNR was maximum (40.18 μV) as compared to W/W (25.67 μV) and R/B (12.88 μV). Fig. 1 shows different PhNR responses among normal compared to diabetic eyes with different severities of DR, and Fig. 2 represents the fundus photo corresponding to different groups along with PhNR responses. [Insert Figs. 1 and 2].

The receiver operating characteristic (ROC) curve was analyzed for all the three color-stimuli (W/W, R/B, and B/Y) PhNR among controls and the diabetic group. The largest area under the curve (AUC) was seen in B/Y PhNR (94%, $P < 0.001$), with maximum sensitivity and specificity of 88% and 87%, respectively. The W/W PhNR also showed an almost a similar AUC (92%, $P < 0.001$) with a sensitivity and specificity of 84% and 81%, respectively. R/B PhNR showed lesser but significant AUC (87%, $P < 0.001$) with a sensitivity and specificity of 74% and 78%, respectively [Table 4]. Fig. 3 represents the ROC curve of PhNR with different color stimuli in the diabetic group.

Discussion

The PhNR is known to predominantly originate from the spiking activity of retinal ganglion cells (RGC).[5-7] Inhibition of electrical activity of the RGCs and amacrine cells was observed in experimental monkeys with induced glaucoma.[5] In patients with DR, the RGCs are at risk of damage due to retinal toxicity with increased glutamate levels, as seen in eyes with diabetes. Earlier studies have noted a reduction of PhNR amplitude with a delay in implicit time in eyes of patients with diabetes.[8-10] In the present study, different chromatic stimuli were used to elicit ERG in subjects with different stages of DR to determine which parameter was most affected in patients with diabetes.

In the present study, the reduction in amplitudes of a-wave, b-wave, W/W PhNR, R/B PhNR, and B/Y PhNR

Table 2a: Amplitudes and implicit times of a-wave in broadband and monochromatic ERG in controls and different severities of diabetic retinopathy

	Broadband		Red on blue		Blue on yellow	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	67.28±12.88	16.72±1.02	50.45±10.03	16.60±0.95	103.95±22.18	17.39±1.55
No DR (*n*=11)	46.00±11.57	17.72±1.45	40.09±8.73	17.84±1.16	48.92±19.01	19.81±2.73
Mild NPDR (*n*=11)	42.22±6.61	21.72±11.22	37.44±8.68	18.63±1.35	48.60±18.78	20.30±2.72
Moderate NPDR (*n*=10)	38.00±7.07	18.91±1.83	36.57±9.44	18.66±2.46	45.11±22.62	20.76±2.62
Severe NPDR (*n*=9)	36.03±10.57	18.93±1.53	32.52±6.67	19.25±0.85	46.81±13.58	22.51±3.63
PDR (*n*=11)	35.77±16.16	20.37±1.95	28.44±12.20	20.11±1.55	42.41±28.94	22.78±2.97
†*F* statistic	27.84,	4.20,	12.52,	19.62,	35.61,	16.35,
P	<0.0001*	<0.002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

DR: Diabetic retinopathy, NPDR: Nonproliferative DR, PDR: Proliferative DR. Statistical ANOVA test was performed, *statistically significant. Bonferroni *post hoc* test with a conservative *P*<0.008

Table 2b: Amplitudes and implicit times of b-wave in broadband and monochromatic ERG in controls and different severities of diabetic retinopathy

	Broadband		Red on blue		Blue on yellow	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	134.39±28.78	38.67±1.71	132.02±23.97	35.16±2.05	195.46±52.46	36.01±2.10
No DR (*n*=11)	138.66±54.48	35.30±2.93	122.48±31.61	40.60±11.62	123.26±48.98	38.06±4.84
Mild NPDR (*n*=11)	125.34±39.17	36.36±3.40	109.86±28.33	37.72±3.89	127.51±37.30	38.72±4.48
Moderate NPDR (*n*=10)	126.09±39.11	38.70±3.96	124.01±40.40	39.62±3.57	114.47±34.05	40.10±6.50
Severe NPDR (*n*=9)	102.59±31.88	37.82±4.10	110.50±29.27	40.45±4.25	104.18±34.27	39.55±3.19
PDR (*n*=11)	88.25±44.50	41.43±4.20	94.40±43.40	41.85±3.90	92.79±47.95	43.39±5.26
†*F* statistic	3.89,	0.85,	3.60,	6.42,	16.40,	1.67,
P	<0.003*	0.51	<0.005*	<0.0001*	<0.0001*	0.14

DR: Diabetic retinopathy, NPDR: Nonproliferative DR, PDR: Proliferative DR, PhNR: Photopic negative response, Sig: Significance. Statistical ANOVA test was performed, *statistically significant. Bonferroni *post hoc* test with a conservative *P*<0.008

Table 2c: Amplitudes and implicit times of PhNR in broadband and monochromatic ERG in controls and different severities of diabetic retinopathy

	Broadband		Red on blue		Blue on yellow	
	Amplitude	Implicit time	Amplitude	Implicit time	Amplitude	Implicit time
Controls (*n*=50)	67.19±16.00	72.56±7.61	53.48±14.15	73.00±5.43	107.68±25.37	101.22±19.46
No DR (*n*=11)	41.51±11.57	74.77±8.00	40.60±11.76	78.21±8.08	64.61±34.94	104.78±22.16
Mild NPDR (*n*=11)	35.42±17.25	76.49±6.60	33.03±13.42	78.48±9.13	57.78±32.57	106.60±24.93
Moderate NPDR (*n*=10)	25.13±14.35	81.17±7.34	22.74±13.89	84.83±10.79	49.04±28.30	95.09±18.22
Severe NPDR (*n*=9)	28.90±12.20	83.24±11.19	25.24±14.57	83.79±7.94	44.75±30.11	99.62±19.25
PDR (*n*=11)	29.39±18.24	87.65±7.89	12.84±9.62	86.17±7.00	40.03±34.49	109.09±0.19.91
†*F* statistic	26.09,	8.92,	23.03,	10.56,	26.21,	0.68,
P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.63

DR: Diabetic retinopathy, NPDR: Nonproliferative DR, PDR: Proliferative DR, PhNR: Photopic negative response. Statistical ANOVA test was performed, *Statistically significant. Bonferroni *post hoc* test with a conservative *P*<0.008

was significant ($P < 0.05$) in the diabetic group as compared to the controls. The reduction of PhNR mean amplitudes in W/W, R/B, and B/Y was greater as compared to a-wave and b-wave amplitude reduction [Tables 1a, 1b, and 1c], suggesting PhNR to be affected more severely by DR. Also, a comparison between the diabetic and control groups showed a maximum reduction in the mean amplitude of B/Y PhNR (52.64 μV) compared to W/W (33.31 μV) and R/B PhNR (24.71 μV) [Tables 1a-1c]. The delay in implicit time was statistically significant in W/W and R/B PhNR ($P < 0.05$) but was not significant in B/Y PhNR ($P = 0.6$). Kim *et al.*[8] also reported delay in PhNR implicit times in eyes with diabetes as compared to controls using W/W ERG. Moreover, because of a marked reduction in B/Y PhNR (more than W/W and R/B PhNR), especially in severe cases, it may be difficult to accurately assess the implicit times.

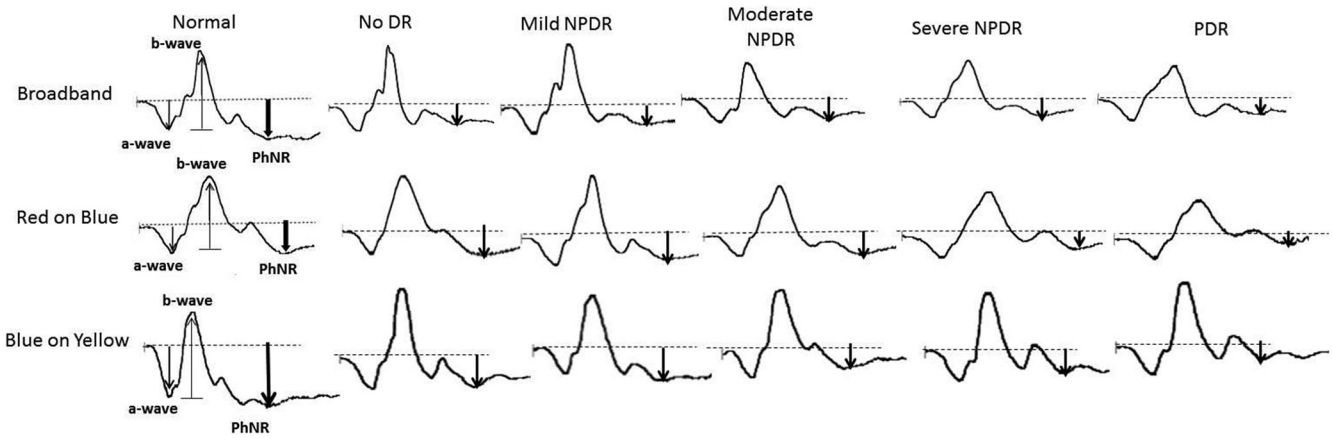

Figure 1: Comparison between normal and diabetic with no clinically visible DR and different severities of DR in W/W, R/B, and B/Y PhNR

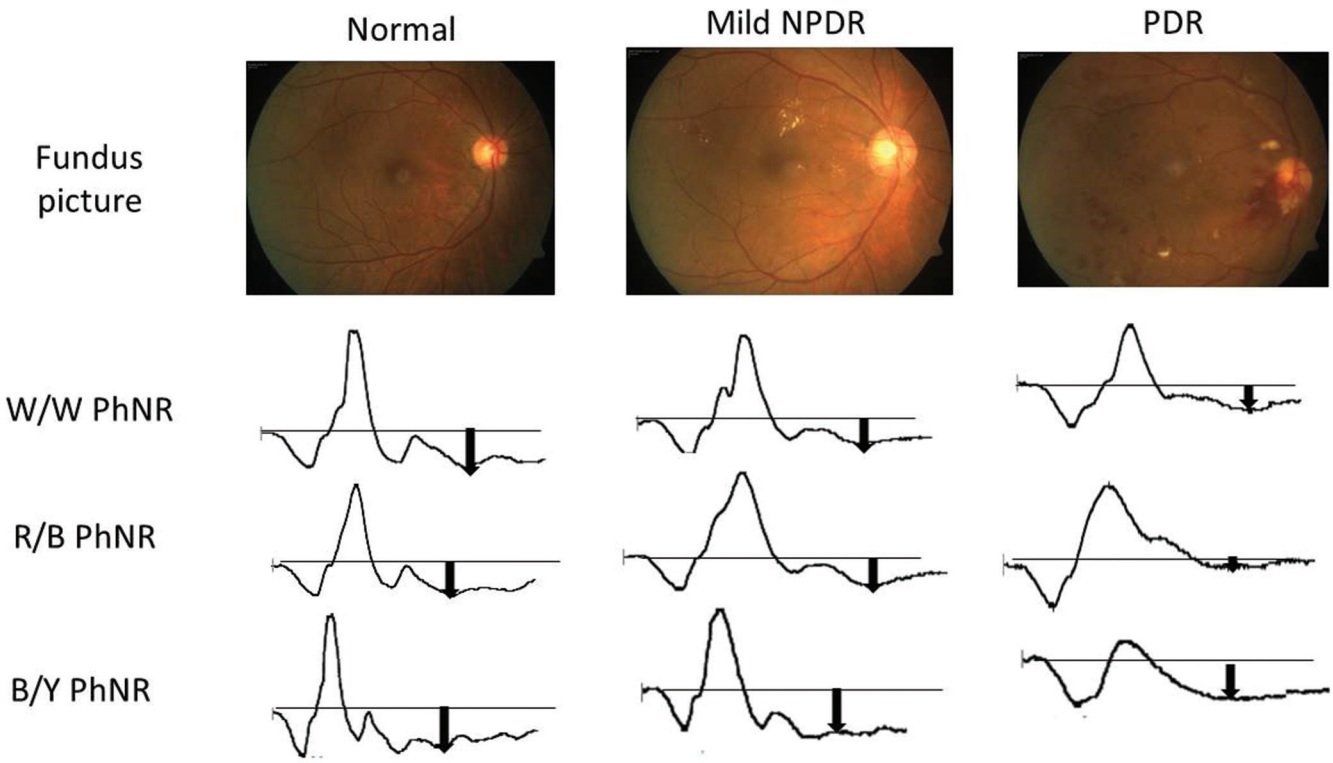

Figure 2: Comparison of fundus photo and PhNR among normal and different severity of diabetic retinopathy with three different color stimuli

We observed a reduction in amplitudes and delay in implicit times of a-wave, b-wave, and three chromatic PhNR (W/W, R/B, and B/Y) in all stages of DR. As the severity of disease increases, a greater decrease in PhNR amplitudes is seen [Tables 2a, 2b, and 2c]. The reduction of B/Y PhNR was consistently higher compared to W/W and R/B PhNR. The mean difference between controls and no DR in B/Y PhNR was 43.07 μV, whereas in W/W PhNR, the difference was only 25.68 μV, and in R/B PhNR, the difference was 12.88 μV [Tables 2a, 2b, and 2c]. *Post hoc* Bonferroni revealed a reduction in PhNR amplitudes even in those with no clinically visible DR compared to controls (supplementary material 2), which somewhat differs from the observations by Park *et al*.[16] who reported a decrease in amplitudes only in the moderate NPDR group.

The B/Y PhNR and W/W PhNR showed highest AUC of 94% and 92%, respectively. Maximum sensitivity and specificity were observed in B/Y PhNR (88% and 87%, respectively) compared to W/W PhNR (84% and 81%, respectively) and R/B (74% and 78%, respectively). The R/B PhNR had a comparatively lower AUC as well (87%). Studies have reported an increased level of glutamate in the vitreous of rats with streptozotocin-induced diabetes.[17,18] Glutamate toxicity in cerebellar granule cells is found to increase neurofilament phosphorylation.[19] Increased phosphorylation of neurofilaments, including in the axons of neurons such as retinal ganglion cells, is considered a feature of neurodegeneration.[20]

Table 3: Mean amplitude reduction in a-wave, b-wave, and PhNR between control and stages of diabetic group

	Controls (*n*=50)	No DR (*n*=11)	Sig	Mild NPDR (*n*=11)	Sig	Moderate NPDR (*n*=10)	Sig	Severe NPDR (*n*=9)	Sig	PDR (*n*=11)	Sig
a-wave	67.28±12.88	46.00±11.57	<0.05*	42.22±6.61	<0.05*	38.00±7.05	<0.05*	36.04±10.57	<0.05*	38.17±12.30	<0.05*
b-wave	134.39±28.78	138.66±54.48	0.71	125.34±39.16	0.38	126.10±39.11	0.43	102.59±31.88	<0.05*	88.25±44.50	<0.05*
Broadband PhNR	67.18±15.99	41.51±11.57	<0.05*	35.42±17.25	<0.05*	25.13±14.35	<0.05*	28.90±12.20	<0.05*	29.39±18.24	<0.05*
Red on Blue PhNR	53.48±14.15	40.60±11.76	<0.05*	33.04±13.42	<0.05*	22.75±13.89	<0.05*	35.24±14.57	<0.05*	12.84±9.62	<0.05*
Blue on Yellow PhNR	104.79±24.37	64.61±34.94	<0.05*	57.78±32.58	<0.05*	49.04±28.30	<0.05*	56.87±20.75	<0.05*	40.03±34.49	<0.05*

DR: Diabetic retinopathy, NPDR: Nonproliferative DR, PDR: Proliferative DR, PhNR: Photopic negative response, Sig: Significance. Unpaired *t*-test was performed, *Statistically significant. Each severity of disease was compared with controls

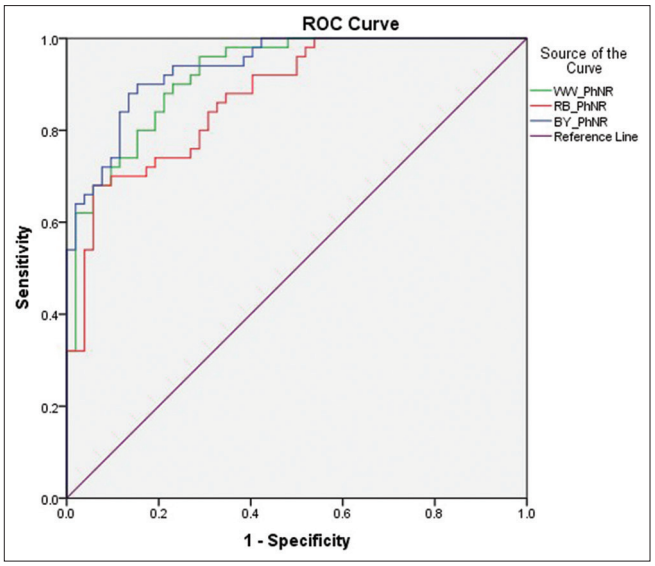

Figure 3: Receiver operating characteristic curve of photopic negative response in diabetic eyes

This reduction in the RGCs is well picked up by more severe affection of the PhNR. Reduction in PhNR amplitude is considered a major biomarker in terms of clinical diagnosis compared to the delay in implicit time.[10] The current study also satisfies the same as the reduction in PhNR amplitude in the diabetic group (especially B/Y PhNR) is much larger (no DR: 43.07 mV, PDR: 67.67 mV) [Table 2c] as compared to the delay in implicit time (3–8 ms delay) [Table 2c].

B/Y PhNR was observed to be a better indicator as compared to W/W PhNR in the present study because of its significant reduction of amplitude even in early stages of DR. Short-wavelength (S-cone) ERG has also been studied in eyes of patients with diabetes and has shown a significant reduction in the amplitudes.[10,21,22] S-cone pathways play an important role in the pathogenesis of DR because of their increased vulnerability. While W/W PhNR elicits responses from all three cone types, especially from the L/M-cone pathways, B/Y has a major contribution from the S-cone pathways. Reportedly, loss of functional integrity of S-cone pathways occurs earlier than the visible vascular changes occurring in patients with diabetes.[23-25] Patients with early diabetes have shown a greater loss in sensitivity of S-cone pathways as compared to L/M-cone.[26] Also, a large and selective decrease in amplitudes of B/Y PhNR may be due to early loss of integrity of the small bi-stratified ganglion cells found only in the S-cone pathways.[10]

This could explain the more severe affection of the B/Y PhNR responses seen in the current study.

The current study had few limitations. The study sample is relatively small. Moreover, the association between PhNR changes with glycemic control has not been observed. Longitudinal studies to look for the effect of diabetes control on PhNR as well as comparison with other functional parameters can be done in future studies with a larger sample size.

Table 4: Receiver operating characteristic (ROC) curve analysis of broadband, red on blue and blue on yellow PhNR amplitudes

	AUC	*P*	Cutoff value	Sensitivity	Specificity
Broadband PhNR			50.10	0.84	0.79
	0.92	<0.001*	50.18	0.84	0.81
			51.17	0.82	0.81
Red on blue PhNR			43.29	0.74	0.75
	0.87	<0.001*	43.73	0.74	0.78
			44.24	0.74	0.81
Blue on yellow		<0.001*	75.74	0.88	0.85
PhNR	0.94		79.87	0.88	0.87
			84.79	0.86	0.87

*Statistically significant. AUC: Area under the curve, PhNR: Photopic negative response

Conclusion

To the best of our knowledge, the comparison of various chromatic stimuli (i.e., W/W, R/B, and B/Y) in various stages of DR has not been previously observed and compared in any study.

This evaluation of ERG parameters in various stages of DR shows us that affection of functional changes in the retina as determined by ERG can occur much before visible diabetic retinopathy. PhNR amplitude can be the single most useful screening tool in patients with diabetes, and the reduction of PhNR amplitude can be considered as a biomarker for the development of DR and can be used to educate patients for better control of diabetes and prevent DR. However, larger studies are necessary to further evaluate its widespread clinical application in the management of diabetic patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy: II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol 1984;102:520-6.
2. Barber AJ. A new view of diabetic retinopathy: A neurodegenerative disease of the eye. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:283–90.
3. Simó R, Hernández C. Neurodegeneration in the diabetic eye: New insights and therapeutic perspectives. Trends Endocrinol Metab 2014;25:23-33.
4. LeCaire T, Palta M, Zhang H, Allen C, Klein R, D'Alessio D. Lower-than-expected prevalence and severity of retinopathy in an incident cohort followed during the first 4–14 years of type 1 diabetes: The Wisconsin Diabetes Registry Study. Am J Epidemiol 2006;164:143-50.
5. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith EL 3rd. The photopic negative response of the macaque electroretinogram: Reduction by experimental glaucoma. Invest Ophthalmol Vis Sci 1999;40:1124-36.
6. Machida S, Tamada K, Oikawa T, Gotoh Y, Nishimura T, Kaneko M, *et al*. Comparison of photopic negative response of full-field and focal electroretinograms in detecting glaucomatous eyes. J Ophthalmol 2011;2011:564131.
7. North RV, Jones AL, Drasdo N, Wild JM, Morgan JE. Electrophysiological evidence of early functional damage in glaucoma and ocular hypertension. Invest Ophthalmol Vis Sci 2010;51:1216-22.
8. Kim HD, Park JY, Ohn YH. Clinical applications of photopic negative response (PhNR) for the treatment of glaucoma and diabetic retinopathy. Korean J Ophthalmol 2010;24:89-95.
9. Chen H, Zhang M, Huang S, Wu D. The photopic negative response of flash ERG in non-proliferative diabetic retinopathy. Doc Ophthalmol 2008;117:129-35.
10. McFarlane M, Wright T, Stephens D, Nilsson J, Westall CA. Blue flash ERG PhNR changes associated with poor long-term glycemic control in adolescents with type 1 diabetes. Invest Ophthalmol Vis Sci 2012;53:741-8.
11. Mortlock KE, Binns AM, Aldebasi YH, North RV. Inter-subject, inter-ocular and inter-session repeatability of the photopic negative response of the electroretinogram recorded using DTL and skin electrodes. Doc Ophthalmol 2010;121:123-34.
12. Sustar M, Cvenkel B, Brecelj J. The effect of broadband and monochromatic stimuli on the photopic negative response of the electroretinogram in normal subjects and in open-angle glaucoma patients. Doc Ophthalmol 2009;118:167-77.
13. Wilkinson CP, Ferris FL III, Klein RE, Lee PP, Agardh CD, Davis M, *et al*. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110:1677-82.
14. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, *et al*. ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 2015;130:1-2.
15. Banerjee A, Khurana M, Sachidanandam R, Sen P. Comparison between broadband and monochromatic photopic negative response in full-field electroretinogram in controls and subjects with primary open-angle glaucoma. Doc Ophthalmol 2019;138:21-33.
16. Park SE, Sun HJ, Lee HJ, Park TK, Ohn YH. The role of electroretinography in assessing the progression of diabetic retinopathy. J Korean Ophthalmol Soc 2010;51:693-9.
17. Lieth E, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D, *et al*. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Penn State Retina Research Group. Diabetes 1998;47:815-20.
18. Lieth E, LaNoue KF, Antonetti DA, Ratz M. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. The Penn State Retina Research Group. Exp Eye Res 2000;70:723-30.
19. Asahara H, Taniwaki T, Ohyagi Y, Yamada T, Kira JI. Glutamate enhances phosphorylation of neurofilaments in cerebellar granule cell culture. J Neurol Sci 1999;171:84-7.
20. Terada M, Yasuda H, Kikkawa R. Delayed Wallerian degeneration and increased neurofilament phosphorylation in sciatic nerves

of rats with streptozocin-induced diabetes. J Neurol Sci 1998;155:23-30.

21. Mortlock KE, Chiti Z, Drasdo N, Owens DR, North RV. Silent substitution S-cone electroretinogram in subjects with diabetes mellitus. Opthalmic Physiol Opt 2005;25:392-9.
22. Kizawa J, Machida S, Kobayashi T, Gotoh Y, Kurosaka D. Changes of oscillatory potentials and photopic negative response in patients with early diabetic retinopathy. Jpn J Opthalmol 2006;50:367-73.
23. Gastinger MJ, Kunselman AR, Conboy EE, Bronson SK, Barber AJ. Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice. Invest Ophthalmol Vis Sci 2008;49:2635-42.
24. Terasaki H, Hirose H, Miyake Y. S-cone pathway sensitivity in diabetes measured with threshold versus intensity curves on flashed backgrounds. Invest Ophthalmol Vis Sci 1996;37:680-4.
25. Bresnick GH, Condit RS, Palta M, Korth K, Groo A, Syrjala S. Association of hue discrimination loss and diabetic retinopathy. Arch Ophthalmol 1985;103:1317-24.
26. Greenstein VC, Hood DC, Ritch R, Steinberger D, Carr RE. S (blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. Invest Opthalmol Vis Sci 1989;30:1732-7.