Original Article

Clinical Application of Photopic Negative Response of the Flash Electroretinogram in Primary Open-angle Glaucoma

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Abstract

Purpose: To evaluate the diagnostic performance of the photopic negative response (PhNR) for the detection of primary open-angle glaucoma (POAG).

Methods: Fifty-two normal subjects (52 eyes) and 173 POAG patients (173 eyes) were studied. The PhNR was elicited using a white stimuli on a white background. The mean deviation (MD) and pattern standard deviation (PSD) of the visual field were measured using standard automated perimetry (SAP). Spectral domain optical coherence tomography (SD-OCT) was used to measure the mean thickness of the retinal nerve fiber layer (RNFL).

Results: In the glaucoma group, as compared to the normal group, the amplitudes of a-waves, b-waves and PhNR were significantly smaller ($P<0.001$), and the PhNR implicit time was significantly longer ($P=0.004$). The MD, PSD and mean thickness of the RNFL were significantly correlated with the amplitude of the PhNR ($P<0.001$). The area under the receiver operating characteristic curve (AUCs) for the amplitudes of a-waves, b-waves and PhNR were 0.853, 0.830 and 0.918, respectively. When the specificity was ≥95%, the sensitivities were 60.4%, 54.2% and 85.4% respectively.

Conclusion: The PhNR amplitude was reduced even when the loss in visual field sensitivity was mild, which suggests that PhNR might be a useful indicator of early glaucoma disease.
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Keywords: electroretinography; primary open-angle glaucoma; photopic negative response

Introduction

Electroretinography (ERG) measures the electrical responses of the retina. A typical pattern of a flash ERG recording includes three individual waves. The a-wave is the first negative potential, mainly originating from the photoreceptor cells[1]. The b-wave is a positive potential that follows the a-wave. It originates from the combined responses of the bipolar cells, horizontal cells and müller cells[2]. Recent studies have shown that the photopic negative response (PhNR), a slow negative potential that follows the b-wave, may be useful for the detection of early glaucoma[4,5]. PhNR represents an electrical signal that originates from the retinal ganglion cells (RGCs) and may reflect the inner retinal function[3]. However, PhNR performance for detecting glaucoma in eyes of Chinese population has not been investigated.

Materials and methods

Study subjects

A total of 173 POAG patients (173 eyes) were as signed into three groups (1) the early group (65 subjects 65 eyes), (2) the advanced group (55 subjects 55 eyes), and (3) the end-stage group (53 subjects 53 eyes) according to the visual field results (as described below). POAG was defined as the intraocular pressure was >21 mmHg together with characteristic glaucomatous optic disc changes and corresponding visual field defects measured by static perimetry, and an open-angle by goniscopy. Secondary glaucoma (e.g. uveitic glaucoma) was excluded. Visual field defects were identified when (1) the pattern deviation plot showed a cluster of ≥3 nonedge points that had sensitivities < the lower 95% centile ranges ($P<0.05$) with at least one less than the lower 99% centile range ($P<0.01$) or (2) the value of the corrected PSD was < the lower 95% centile range ($P<0.05$); or (3) the Glaucoma Hemifield Test was outside the normal limits.

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Fifty-two eyes of 52 age-matched normal volunteers (aged from 20 to 73 years, 40.0±13.6 years on average) were included. Participants had no medical or ocular diseases and had a best-corrected visual acuity of 20/20. Subjects who had myopia < 6.0 diopters (D) were excluded.

ERG recordings

The photopic ERGs were elicited by white stimuli of 2cd·s/m^2 on a white background of 25 cd/m^2. This recording condition conformed to the standard for clinical ERG[7]. The stimulus and background lights were produced by light-emitting diodes (LEDs). Before the recordings, all subjects were light adapted to the background light for at least 10 minutes. The pupils were maximally dilated to 8.0 mm by 1% tropicamide. After light adaptation, a corneal electrode was applied under topical anesthesia with 0.5% proparacaine(Alcaine®; Alcon-Couvreur, Puurs, Belgium). One percent methyl-cellulose was spread over the surface of the electrode to prevent mechanical damage to the cornea. The reference electrode was placed on the lateral canthus, and a ground electrode was attached to the center of the forehead. The duration of the stimulation was limited to less than five milli-seconds (ms) and the intensity and duration were controlled by an electronic stimulator. The interval between the stimuli was greater than two seconds. Electrical signals from the electrode were amplified 1000 times and were digitalized using an analog-to-digital converter. High and low cutoff limits for the signals were set at 300 Hz and 1.0 Hz, respectively. The averaged results from five repeated measurements were analyzed in this study.

The a- and b-wave amplitudes were measured from the baseline to the trough of the first negative response and from the first trough to the peak of the following positive wave, respectively. The PhNR amplitude was defined as the difference between the baseline and the peak of the negative wave following the b-wave. The PhNR implicit time was defined as the interval between the stimulation and the peak of the negative wave. A single experienced technician measured the peak point of the wave. The mean amplitudes and mean implicit times of the PhNR, and the a- and b-wave amplitudes were compared among the normal and the glaucoma.

Visual field analyses

The Humphrey Visual Field Analyzer (Model 750-II, Humphrey Instruments, Carl Zeiss Meditec, Dublin, CA, USA) SITA standard 24-2 strategy was used. Patients were classified into three groups[8] (1) early (MD > -6 dB; n=65, mean age ± SD: 47.6±15.0 years); (2) moderate (–6 dB≥MD≥12 dB; n=55, mean age ± SD: 46.8±17.8); and (3) advanced (MD<12 dB; n=53, mean age ± SD: 42.6±16.5).

When the fixation losses were >20%, the false-positive and false-negative rates > 15%, the visual field was considered to be unreliable and excluded from the analysis. The interval between the visual field testing and ERG recording was less than 1 month.

SD-OCT

The RNFL was imaged by the Cirrus HD-OCT (software version 5.0, Carl Zeiss Meditec, Dublin, California) using the optic disc cube scan. It is composed of 200×200 A-scans covering an area of 6×6 mm^2 centered on the optic nerve head.The total scan time was 1.48 seconds. Scans with movement artifact or signal strength of less than 6 were excluded from the analysis.

Statistical analysis

The area under the receiver operating characteristic curve (AUC) was used to measure the diagnostic ability to differentiate glaucomatous from normal eyes. The PhNR, MD, PSD and RNFL measurements between the normal and glaucoma groups were compared with One-way ANOVA. Pearson's coefficient of correlation was calculated to determine the degree of correlation between PhNR and MD, PSD, RNFL measurements. In all statistical analyses, $P<0.05$ was considered statistically significant. All analyses were performed using commercial software SPSS 17.0.

Results

Comparison between the normal and POAG groups

The mean age, refractive error, and gender distribution did not differ between the normal and POAG groups (Table 1).

In the normal group, the mean amplitude of the a-wave and the b-wave was 44.8±6.4 uV, 166.6±33.1

Table 1 Demographic and clinical data for this study

Years	Normal	POAG			*P* values(ANOVA analysis)
		Early	Advanced	End-stage	
Gender(M/F)	40.0±13.6	47.6±15.0	46.8±17.8	42.6±16.5	0.081
Refractive error (Diopter)	23/29	33/32	26/29	26/27	0.074
Amplitude(μv)	−2.9±1.9	−2.8±2.4	−3.0±2.5	−4.1±2.8	0.055
a-wave amplitude	44.8±6.4	32.2±9.6	29.6±8.1	30.4±7.8	0.000
b-wave amplitude	166.6±33.1	121.4±35.4	111.6±29.7	128.8±28.3	0.000
PhNR amplitude (uV)	50.1±6.4	29.5±6.0	23.1±9.0	17.5±7.3	0.000
PhNR implicit time (ms)	66.4±3.6	67.1±4.5	68.7±4.8	69.1±4.7	0.004

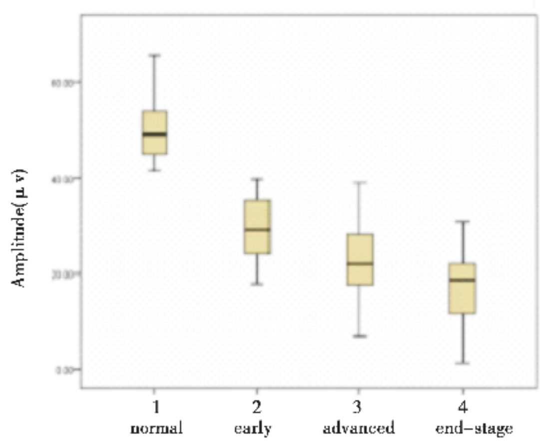

Figure 1 PhNR amplitude

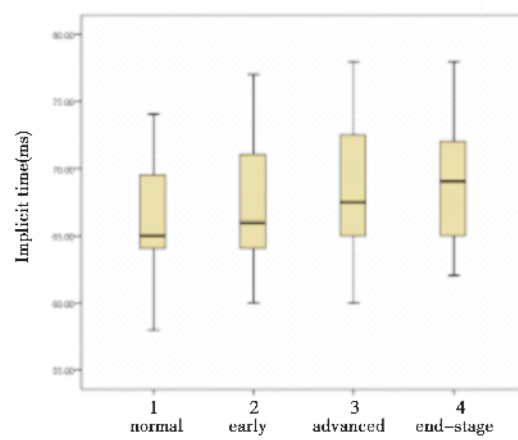

Figure 2 PhNR implicit time

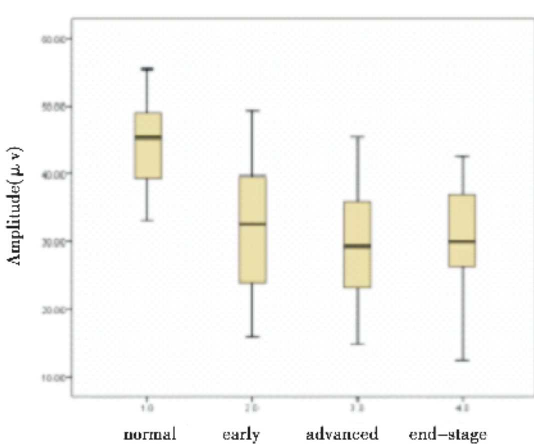

Figure 3 a−wave amplitude

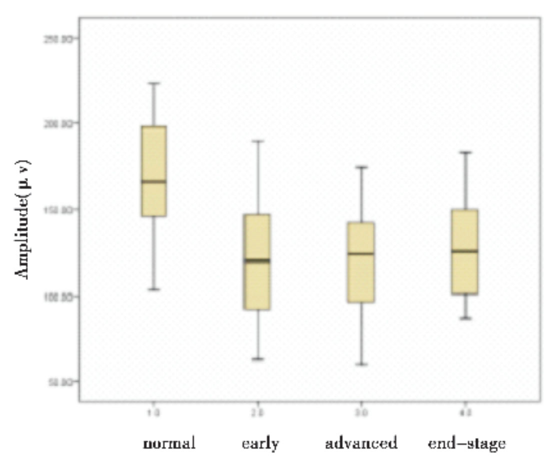

Figure 4 b−wave amplitude

Figures 1,2,3,4 Comparison of a-waves, b-waves and photopic negative responses (PhNRs) among the four groups

uV, respectively. The mean amplitude of the PhNR was 50.1±6.4 uV, and the mean implicit time was 66.4±3.6 ms.

In the early POAG group, the mean a-wave and the b-wave amplitude were at 32.2±9.6 uV, 121.4±35.4 uV, respectively. The mean amplitude of the PhNR was 29.5±6.0 uV, and the mean implicit time was 67.1±4.5 ms.

In the moderate POAG group, the mean a-wave and the b-wave amplitude were 29.6±8.1 uV, 111.6±29.7 uV, respectively. The mean amplitude of the PhNR was 23.1±9.0 uV, and the mean implicit time was 68.7±4.8 ms.

In the advanced POAG group, the mean a-wave and the b-wave amplitude were measured at 30.4±7.8 uV, 128.8±28.3 uV, respectively. The mean amplitude of the PhNR was 17.5±7.3 uV, and the mean implicit time was 69.1±4.7 ms.

The average a-wave and b-wave amplitudes were significantly smaller in glaucomatous eyes than those in the normal eyes (P=0.000). However, there was no significant difference in the a-wave and b-wave amplitudes among the glaucoma groups(P > 0.05).

The mean amplitude and implicit time of the PhNR were significantly reduced in the POAG compared to that of the normal group (P=0.000 and 0.004). In addition, there was a significant difference in the PhNR amplitude and the implicit time of the PhNR among the glaucoma groups(P < 0.05).

Correlation between the PhNR and other parameters

MD and mean RNFL thickness showed significant positively correlations with the PhNR amplitude (P< 0.001, R^2=0.59, 0.45, respectively)(Table 2, Figures 5, 6). PSD showed a weak, but significant correlation with the PhNR amplitude (P=0.016, R^2=-0.37) (Table 2, Figure 7). However, the MD, PSD and mean RNFL thickness had no correlation with the amplitude of a-wave and b-wave or the PhNR implicit time(P>0.05)(Table 2).

Table 2 The correlation coefficient between the PhNR and other parameters

Parameters	PhNR amplitude		PhNR implicit time		a–wave amplitude		b–wave amplitude	
	R^2	P values	R^2	P values	R^2	P values	R^2	P values
MD	0.59	0.000	–0.23	0.138	0.02	0.926	–0.11	0.498
PSD	–0.37	0.016	0.24	0.125	–0.14	0.395	–0.28	0.081
mRNFL thickness	0.45	0.000	–0.10	0.549	–0.08	0.626	–0.05	0.742

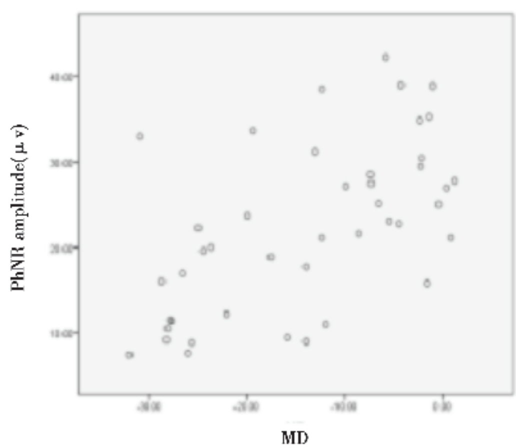

Figure 5 The scatter plots of PhNR amplitude and MD

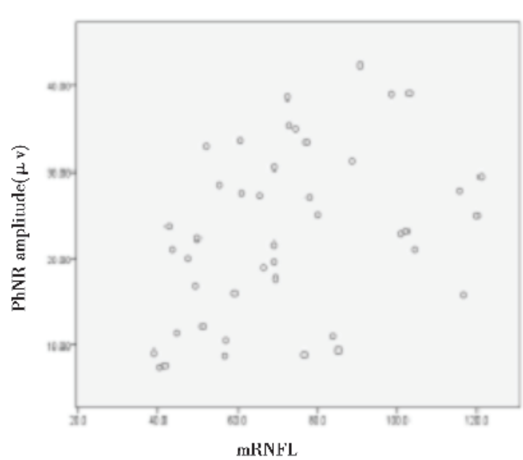

Figure 6 The scatter plots of PhNR amplitude and mRNFL

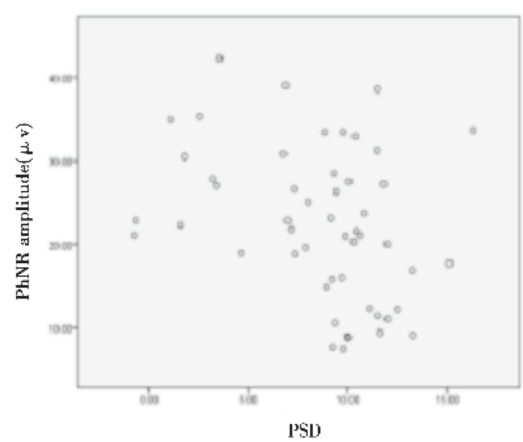

Figure 7 The scatter plots of PhNR amplitude and PSD

Sensitivity and specificity of the PhNR in early POAG

Figure 8 shows the ROC curves for PhNR, a-wave and b-wave amplitudes. The area under the curves (AUC) was largest (0.918) for the PhNR amplitude and smallest (0.830) for the b-wave amplitude (Table 3, Figure 8). There were significant differences in the AUCs between the PhNR amplitude and the a-wave, b-wave amplitudes (P=0.000, Table 3).

When the specificity was ≥95%, the sensitivity for PhNR, a-wave and b-wave amplitudes was 85.4%, 60.4% and 54.2%, respectively. The sensitivity for the PhNR amplitude was significantly high-

Table 3 The ROC curves analysis between the early POAG group and normal group

Parameters	AUC(95%CI)	Sensitivity Specificity≥95%	*P* values
a-wave amplitude	0.853(0.779±0.927)	60.4	0.000
b-wave amplitude	0.830(0.750±0.910)	54.2	0.000
PhNR amplitude	0.918(0.851±0.985)	85.4	0.000

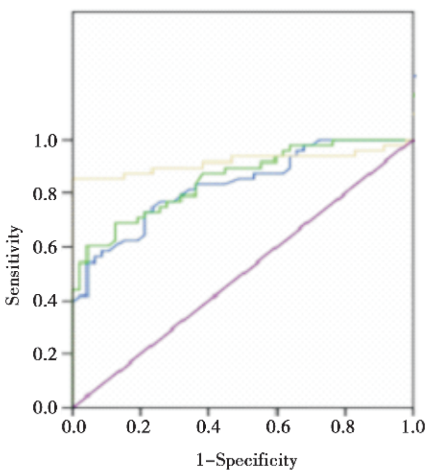

Figure 8 Receiver operating characteristic (ROC) curves for PhNR, a- and b-wave amplitudes

er than other parameters (Table 3, *P*<0.05).

Discussion

Glaucoma is characterized by progressive degeneration of the optic nerve and loss of retinal ganglion cells (RGCs)[12]. RGCs have voltage-gated sodium channels, allowing them to take part in the generation of an electrical potential via the Na^+–K exchange. Viswanathan et al[3]. compared the ERG findings between normal eyes and tetrodotoxin-injected eyes in monkeys. Tetrodotoxin inhibits the electrical activity of RGCs and amacrine cells. The results of their study showed that the PhNR amplitude was reduced and the implicit time was prolonged in the tetrodotoxin-injected eyes. In addition to this experimental evidence, it has been demonstrated that the PhNR was reduced in patients with optic nerve and retinal diseases that affect mainly the RGCs and retinal nerve fiber layer[13,14]. In our study, the PhNR amplitude was reduced and the implicit time was prolonged in the POAG group. These findings are similar to those reported by other studies[5]. However, some findings in our study were different from the past studying results. The a-wave and b-wave amplitudes were also significantly reduced in the POAG group compared to those of the normal group. Colotto et al[15]. noted that the amplitudes of the a-wave and b-wave were not significantly different between POAG patients and normal subjects, and that there was no difference in PhNR implicit time between the POAG and normal groups.

The differences may be related to the following: First, the difference of sample size and the selection of the patients. Sample size in our study is larger than the past study. Including of the early, advanced and end-stage POAG patients, especially end-stage POAG patients, in which the functions of photoreceptor cells, Müller cells, and bipolar cells may be also damaged. So the amplitudes of the a-wave and b-wave in POAG patients were significantly lower than that of normal subjects. Second, the different setting of PhNR stimuls parameters may also lead to different study results. For instance, light intensity, stimuls light and background light are different. For example, Viswanathan et al[3]. using red stimuli on blue background to record PhNR. In contrast, our PhNR was elicited by white stimuli on a white background. At the same time, stimuls light intensity was also different in above two research. Third, the difference of electrode including of electrode type and electrode position. Viswanathan et al[4]. using DTL electrode to record PhNR; Hoon et al[5]. reported that the ground electrode was placed on the earlobe ipsilateral to the tested eye, and a reference electrode was attached to the center of the forehead. These researches differed from those of the present study.

Our study found that the AUC of the PhNR amplitude was superior to detect eyes with early glaucoma compared with the a-wave and b-wave amplitudes. When the specificity was ≥95%, the sensitivity for the PhNR amplitude was significantly higher than the other parameters. These findings indicate that the PhNR amplitude may be a useful indicator to detect early functional changes in glaucoma.

This study indicates that the PhNR is useful to measure early functional loss of RGCs in glaucoma. The PhNR amplitude has a high diagnostic ability to detect early glaucoma.

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