

Pattern Electroretinogram Detects Localized Glaucoma Defects

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Received: 15 December 2017

Accepted: 19 June 2018

Published: 13 September 2018

Keywords: glaucoma; localized defect; electroretinogram; hemifield; hemiretina

Citation: Salgarello T, Giudiceandrea A, Calandriello L, Marangoni D, Colotto A, Caporossi A, Falsini B. Pattern electroretinogram detects localized glaucoma defects. *Trans Vis Sci Tech.* 2018;7(5):6, <https://doi.org/10.1167/tvst.7.5.6>
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Purpose: We evaluated the clinical ability of pattern electroretinogram (PERG) to detect functional losses in the affected hemifield of open-angle glaucoma patients with localized perimetric defects.

Methods: Hemifield (horizontally-defined) steady-state PERGs (h-PERGs) were recorded in response to 1.7 c/deg alternating gratings from 32 eyes of 29 glaucomatous patients with a perimetric, focal one-hemifield defect, 10 eyes of 10 glaucomatous patients with a diffuse perimetric defect, and 18 eyes of 18 age-matched normal subjects. Standard automated perimetry (SAP) and spectral-domain optical coherence tomography (SD-OCT) for retinal nerve fiber layer (RNFL) thickness also were performed. h-PERG amplitudes and ratios, calculated corresponding hemifield perimetric deviations, as well as hemiretina RNFL thicknesses were analyzed.

Results: h-PERG amplitudes, perimetric deviations, and RNFL thicknesses showed losses ($P < 0.001$) when comparing affected with unaffected hemifields of localized glaucomatous eyes. No differences were found in h-PERG amplitudes between hemifields of normal or diffuse glaucomatous eyes. h-PERG amplitude ratios (affected/unaffected hemifield) in localized glaucoma were lower ($P < 0.001$) than the ratios from normal or diffuse glaucomatous eyes. The areas under the receiver operating characteristic curves for h-PERG amplitude ratios, comparing localized-defect glaucomatous eyes with normal or diffuse glaucomatous eyes, were 0.93 and 0.91, respectively.

Conclusions: h-PERG assessment showed good diagnostic accuracy to confirm localized glaucomatous defects detected perimetrically. This test may be particularly useful in cognitively impaired patients or young/nonverbal patients unable to provide reliable visual fields.

Translational Relevance: h-PERG provides a sensitive objective measure to confirm focal losses detected with SAP and/or RNFL thickness analysis.

Introduction

Accurate diagnosis of chronic glaucoma is a mainstay in the management of disease, because of the absence or paucity of clinical symptoms and potential progression of optic nerve damage. The current approach is based on morphologic and functional detection of retinal ganglion cell (RGC) losses by computerized optic nerve head analysis, for example, optical coherence tomography (OCT) and

confocal scanning laser ophthalmoscopy, as well as standard automated perimetry (SAP).¹⁻³

A considerable RGC loss may develop before the manifest glaucoma signs,^{4,5} and can be missed at SAP. Therefore, electrophysiologic techniques may help identify subclinical functional deficits.⁶⁻¹⁰ The electroretinogram (ERG) obtained in response to alternating gratings or checkerboards (pattern ERG [PERG]), an electrical response generated by RGCs,^{11,12} can be used to diagnose and manage patients with suspected or early glaucoma.^{6,7,9,10,13-16}

As it is used currently in the clinic, the PERG represents the integrated electrical response of the stimulated retina. However, glaucoma may affect focal areas of the optic nerve head, more easily detectable by optic nerve imaging and perimetric techniques.^{17–24} Early glaucomatous defects tend to involve the inferotemporal and superotemporal disc regions.²⁵

Two studies^{26,27} reported the results obtained by recording hemifield PERGs (h-PERGs) in patients with focal glaucomatous damage or at risk for glaucoma. h-PERG analysis showed promising results for detection of localized losses in glaucoma. However, previous studies indicated that PERG may detect ganglion cell dysfunction undetected by conventional perimetry,²⁸ raising the possibility that PERG losses may be highly sensitive but not very specific for detecting ganglion cell losses confined to one hemifield.

Assessment of PERG specificity for localized ganglion cell losses may be of clinical relevance. Indeed, if specific, the h-PERG may be an easily implementable tool, used in combination with conventional perimetry and morphometric thickness measures, to confirm the diagnosis of focal glaucomatous defects.

We evaluated the ability of a clinical h-PERG protocol, recording responses from upper and lower retinal hemifields, to detect functional losses in glaucoma patients with a localized perimetric defect in only one hemifield.

Methods

Study Population

We studied 29 patients with primary open-angle glaucoma (65.5% men and 34.5% women; mean age \pm SD, 62.9 \pm 13.4 years; age range, 33–83; 32 eyes) showing a localized one-hemifield defect. A second group of 10 primary open-angle glaucoma patients (50% men, 50% women; mean age, 61 \pm 14.8 years; age range, 39–80; 10 eyes) with evidence of a diffuse perimetric defect of variable severity (mean deviation [MD] range, -2.5 to -9 dB), that is, involving both upper and lower hemifield, also was evaluated for PERG recordings as a comparison group. All patients were enrolled consecutively from a larger cohort of 921 patients evaluated at the Glaucoma Service of the Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore of Roma, Italy, from November 2013 to July 2014. The

normal control group consisted of 18 normal subjects, whose sex and age distribution were comparable with those of patients (44.4% men, 55.6% women; mean age, 60.8 \pm 15.8 years; age range, 34–82; 18 eyes). All patients and normal subjects underwent a full ophthalmologic examination, including Snellen visual acuity (VA) measurement, Goldmann applanation tonometry, computerized white-on-white 30-2 visual field testing by Humphrey Field Analyzer 750i (HFA; Carl Zeiss Meditec, Inc., Dublin, CA) and PERG recording (Steady-state PERG protocol; CSO, Florence, Italy). In patients, gonioscopy and spectral-domain OCT (SD-OCT) analysis (Cirrus HD-OCT; Carl Zeiss Meditec, Inc.) of the retinal nerve fiber layer (RNFL) also were performed. Visual field, PERG, and OCT analysis were obtained for each patient within 1 week of each other. Patients met the following inclusion criteria: elevated intraocular pressure (IOP) at diagnosis (>21 mm Hg on two separate occasions, with at least one daily curve), normal range central corneal thickness values (520–570 μm , as measured by the digital ultrasonic pachymeter Altair V4; Optikon 2000, Rome, Italy), open anterior chamber angle, abnormal optic disc, and abnormal SAP with a focal defect in one hemifield, with normal opposite field, or a diffuse defect involving upper and lower hemifields to a comparable extent (Glaucoma Hemifield Test within normal limits), in at least one eye. Abnormal clinical appearance of the optic disc on routine stereoscopic examination with slit-lamp biomicroscopy and 78-diopter (D) lens was defined by the presence of at least one of the following glaucomatous abnormalities: vertical cup/disc diameter ratio >0.6 (or an interocular cup-to-disc ratio asymmetry ≥ 0.2 , unexplained by side differences in disc size), excavation, thinning of the rim, notching. Abnormal HFA central 30-2 perimetry was defined as a typical reproducible defect (arcuate and/or paracentral scotoma or nasal step) in three consecutive exams,²⁹ with one or more of the following alterations: Glaucoma Hemifield Test outside normal limits, pattern standard deviation (PSD) with $P < 5\%$, a cluster of ≥ 3 adjacent points, not contiguous with the field borders nor the blind spot, in the upper or lower hemifield of the total and pattern deviation plots with $P < 5\%$, one of which reached $P < 1\%$. This latter feature, independent of the pathologic level, was mandatory for each eye with localized defect to be included into the study, together with the lack of points with a $\leq 5\%$ probability level on total and pattern deviation plots in the opposite hemifield. By contrast, all patients with a diffuse



Figure 1. Typical h-PERG recording in the clinical setting, by stimulating the upper hemifield. The subject fixates at a mark placed midway of the two hemifields.

perimetric defect had to have a normal Glaucoma Hemifield Test confirmed in at least three separate tests. All patients were under treatment by one or more topical hypotensive drugs (β -blockers, prostaglandin analogues, carbonic anhydrase inhibitors, and α_2 -agonists), which were started at least 3 months before patient recruitment and remained unchanged throughout the study, providing a stable IOP lower than 21 mm Hg; no putative neuroprotective agents (i.e., citicoline, epigallocatechin gallate) were used. Exclusion criteria were best corrected VA $<20/25$, refractive error $> \pm 3.00$ D spherical equivalent, astigmatism $> \pm 1.00$ D, previous ocular surgery or trauma, presence of cataract, retinal or neuro-ophthalmologic diseases affecting visual function, optic disc pallor exceeding cupping or low perimetric reliability.³⁰

Written, informed consent from every patient or normal subject, after the procedures used in the study were fully explained, was obtained. The research followed the tenets of the Declaration of Helsinki and was approved by the institutional review board.

Apparatus and Procedure

ERG Recording

Figure 1 shows the typical clinical setting for the h-PERG assessment. In this study, PERG was recorded in response to horizontal square-wave gratings of 1.7 cycles/deg spatial frequency, modulated in square-wave counterphase at 15 reversals/s and electronically generated on a high-resolution television (TV) monitor (contrast, 70%; mean luminance, 80 cd/m²).

Subjects fixated at the midpoint of the border (superior or inferior during the inferior or superior stimulation, respectively) of stimulating field (size, 24° width \times 12° height) with natural pupils, whose size was measured (mean value, 3.5 ± 1.0 mm), at a viewing distance of 57 cm wearing full refractive correction. Fixation was monitored by a trained observer. No statistically significant differences in pupil size were observed between patients and normal subjects. The superior and inferior hemiretinas were stimulated separately, according to the instrument option. The field tested first was randomly selected for each patient. The unmodulated, gray uniform hemifield was kept at the same mean luminance of the stimulating field. ERGs were recorded by an Ag-AgCl electrode taped on the skin of the lower eyelid.^{9,31} A similar electrode, placed below the eyelid of the contralateral unstimulated eye, was used as reference (interocular recording).¹² Ground electrode was on the forehead. Electrode impedances were maintained <5 Kiloohms.

Responses were amplified, filtered (0.3–100 Hz, 6 dB per octave), sampled at 2 kHz with a resolution of 12 bits, and averaged (250 events) with automatic artifact rejection. Amplitude (in μ V) of the Fourier analyzed response second harmonic (i.e., the reversal frequency) was measured. Response phase also was measured. However, previous experience in our lab suggested that PERG phase is not a sensitive indicator of early glaucomatous damage.³² Therefore, it was not included among the main outcome measures of the study. The noise level was estimated after each recording, taken as the difference between odd and even recordings for each run. The noise at the second harmonic was comprised between 0.07 and 0.12 μ V. All responses had a signal-to-noise ratio ≥ 5 . The total h-PERG testing time per eye was ≤ 8 minutes.

For each run, the ratio of h-PERG second harmonic amplitude values (affected hemifield-to-unaffected hemifield of localized glaucoma patients and upper hemifield-to-lower hemifield of controls, either with diffuse glaucoma or normal) was calculated.

Perimetry

Visual field sensitivity was determined for each eye using the HFA central 30-2 SITA-standard test. Only field exams with good reliability indices (fixation losses, false-positive and -negative errors $<20\%$)³³ were evaluated. Definition of normal and abnormal visual fields has been described above (see “Study

Population”). For data analysis, the two global indices of field sensitivity, MD and PSD, were collected, and the hemifield perimetric mean deviation was calculated from the total deviation plot in an area approximately corresponding to that evaluated by the h-PERG. More specifically, the average of the deviations for either upper or lower hemifield was calculated in a rectangular area sized $30^\circ \times 15^\circ$ above or below the field horizontal axis (17 points per hemifield).

OCT Recording

OCT imaging was performed on the patients using the HD-OCT Cirrus (software version 4.0.1) on the peripapillary RNFL. The OCT lens was adjusted for the patient’s refractive error. The subject was instructed to stare at the internal fixation target with the eye under examination, to enable the optic disc to come into the window and to be centered successively. The scan protocol was the Optic Disc Cube 200×200 . After optimizing the reflective signal, three separate scans were obtained per eye during the same session, and the best one with optimal signal strength ($>6/10$) and scan image centering, no movements during scans or anomalous RNFL internal/external boundary definition was used for the analysis. The superior or inferior RNFL thickness was calculated by averaging the thickness values obtained in some corresponding regions, including three supero- or inferotemporal clock hours (globally equivalent to 90° each) of the calculation circle, respectively. These peripapillary regions were chosen because of receiving RGC axons from the PERG-stimulated areas. Values at the 9-o’clock position in right eyes and 3-o’clock position in left eyes were not considered, as they may not be ascribable to a single vertical hemifield.

Statistical Analysis

In the localized glaucoma group, only one eye per patient showed a focal one-hemifield perimetric defect and was included in the analysis, except for three patients in whom both eyes were eligible, on the assumption that this did not affect substantially the main outcome of the study. In the diffuse glaucoma and normal groups, only one eye, randomly selected, was included in the study.

Preliminary recordings indicated that in normals or diffuse glaucoma patients no significant differences were found between upper-to-lower or lower-to-upper h-PERG amplitude ratios ($t = 0.16$, $P = 0.88$). Thus,

the ratios always were computed by dividing upper over lower responses (see also Results).

The data were not normally distributed (Shapiro-Wilk test, $P > 0.05$) and, accordingly, the analysis was done by using nonparametric tests. Given the significant departure from normality, at the individual level we could not use the 95% confidence interval as a cutoff to determine the percentage of abnormal results. We could use, instead, the percentile range and receiver operating characteristic (ROC) curves. A Wilcoxon test was used to separately compare the h-PERG amplitudes, perimetric decibel-based mean deviations, and the OCT RNFL thicknesses between the opposite hemifields of the involved study population groups. Additional analysis by a Kruskal-Wallis test was performed by comparing the amplitude ratios of affected/unaffected h-PERG in localized glaucoma patients with the ratios between upper and lower h-PERG in controls, either diffuse glaucoma patients or normals (between-group comparisons were performed by a Mann-Whitney U test with a conservative P value of <0.01).

To determine the diagnostic accuracy of h-PERG amplitude ratios to differentiate localized glaucomatous from normal eyes, as well as localized versus diffuse glaucomatous eyes, ROC curves were obtained, and the areas under the curve (AUCs) were calculated. In all analyses, $P < 0.01$, to compensate for multiple comparisons, was considered statistically significant.

Results

In patients, the maximum and treated IOP values (i.e., at diagnosis and at study enrollment, respectively; average \pm SD) were 24.56 ± 2.88 (range, 22–33) and 15.28 ± 2.57 (range, 10–20) mm Hg, respectively, for localized glaucoma and 24.4 ± 2.12 (range, 22–28) and 12.9 ± 1.37 (range, 10–15) mm Hg, respectively, for diffuse glaucoma. Main demographic and clinical data of individual patients, including functional (perimetric and electrophysiologic) and OCT findings, are reported in [Tables 1](#) and [2](#) for localized and diffuse glaucomatous eyes, respectively. Individual demographic and electrophysiologic data of normal subjects are reported in [Table 3](#).

In the localized glaucoma group, an arcuate defect at different stages was reported in 29 eyes (51.7% upper and 48.3% lower hemifields), and a nasal step in three eyes (two upper and one lower hemifields). Median perimetric MD and PSD indexes in these eyes were -4.5 dB (range, -1.19 to -11.29) and 5.1 dB

Table 1. Demographic and Clinical Characteristics of Localized Glaucoma Patients

Patients #	Sex	Age (yrs)	Eye	Treated		Affected Hemifield	Unaffected Hemifield	Affected Hemifield	Unaffected Hemifield	Affected Hemifield	Unaffected Hemifield	
				IOP (mm Hg)	Perimetric MD (dB)	Perimetric PSD (dB)	Total Deviation (dB)	Total Deviation (dB)	PERG Amplitude (μV)	PERG Amplitude (μV)	RNFL Thickness (μm)	RNFL Thickness (μm)
1	M	83	LE	13	-3.52	2.95	-5.47	-2.47	0.29	0.53	70	96
2	F	58	RE	15	-6.39	9.91	-8.47	-1.29	0.21	0.60	60	104
3	M	58	LE	15	-2.15	2.23	-3.41	-1.41	0.58	0.65	86	129
4	M	72	RE	12	-9.77	14.32	-22.7	-1.11	0.44	0.54	90	90
5	M	72	LE	14	-4.89	8.94	-6.47	-1.82	0.55	0.84	66	90
6	F	72	RE	17	-5.62	3.70	-6.70	-2.35	0.89	1.06	76	84
7	F	33	RE	14	-3.58	3.55	-4.41	-1.41	0.60	0.88	52	89
7	F	33	LE	13	-5.58	5.05	-8.47	-2.52	0.21	1.08	47	81
8	M	68	RE	10	-7.57	11.24	-11.82	-2.11	0.31	0.47	54	90
8	M	68	LE	10	-9.36	15.05	-20.59	0.23	0.37	0.37	42	72
9	M	75	RE	15	-3.02	3.12	-4.35	-1.59	0.31	0.45	69	84
10	M	75	LE	18	-5.38	5.56	-6.76	-2.23	0.19	0.52	59	79
11	F	45	RE	17	-4.72	5.14	-3.56	-2.91	0.90	0.94	72	86
12	M	76	RE	13	-2.23	2.73	-3.82	-1.76	0.39	0.48	83	109
13	M	62	RE	18	-8.27	11.33	-19.82	-1.76	0.29	0.36	47	64
14	F	76	RE	15	-4.95	2.87	-5.41	-2.41	0.24	0.48	59	105
15	M	61	LE	14	-2.84	2.30	-3.71	-2.59	0.15	0.67	61	80
16	M	72	RE	17	-3.99	2.56	-5.35	-2.29	0.14	0.43	72	84
17	M	67	LE	16	-3.84	2.63	-3.94	-2.71	0.47	0.65	50	78
18	F	75	LE	12	-7.57	11.57	-16.65	-1.12	0.63	0.84	62	109
19	M	67	LE	15	-4.24	6.30	-5.06	-2.06	0.39	0.93	48	88
20	M	64	LE	13	-2.15	2.61	-2.65	-1.29	0.49	0.52	52	96
21	F	70	RE	19	-6.32	7.46	-7.65	-3.06	0.34	0.64	65	90
22	F	52	LE	17	-3.91	2.57	-4.24	-2.82	0.57	0.80	58	88
23	F	68	LE	20	-1.44	1.93	-2.12	-0.24	0.40	1.11	54	85
24	M	71	LE	16	-1.19	1.81	-1.00	-0.82	0.59	0.81	68	88
25	M	66	RE	20	-1.85	3.62	-2.53	-1.18	0.90	1.08	76	95
26	F	46	LE	14	-4.70	10.90	-12.59	0.24	0.80	1.94	79	91
27	M	34	RE	17	-4.30	6.55	-6.71	0.24	0.42	1.02	58	95
28	F	50	RE	17	-11.29	17.56	-28.18	0.35	0.62	0.80	53	84
28	F	50	LE	15	-3.51	5.62	-4.94	-1.24	0.60	1.00	58	94
29	M	73	LE	18	-5.04	7.88	-8.53	-1.18	0.59	1.05	58	105

M, male; F, female; LE, left eye; RE, right eye.

(range, 1.81–17.56), respectively. In the two horizontally-defined hemifields, the medians of the calculated decibel-based mean deviations were -5.44 (range, -1 to -28.18) and -1.68 (range, 0.35 to -3.06) dB in the perimetrically affected and unaffected hemifields, respectively (significant difference, $P < 0.001$). In the diffuse glaucoma group, the median perimetric MD and PSD indexes were -5.07 (range, -2.46 to -8.73) and 2.73 (range, 2.07–14.92) dB, respectively.

In the two horizontally-defined hemifields, the medians of the calculated decibel-based mean deviations were -4.85 (range, 1.12 to -11.59) and -3.8 (range, -1.35 to -9.71) dB in the upper and the lower hemifields, respectively (not significantly different).

In localized glaucomatous eyes, the median h-PERG amplitudes were 0.43 (range, 0.9–0.14) and 0.74 (range, 1.94–0.36) μV in the perimetrically-affected and unaffected hemifields, respectively (sig-

Table 2. Demographic and Clinical Characteristics of Diffuse Glaucoma Patients

Patients #	Sex	Age (yrs)	Eye	Treated IOP (mm Hg)	Perimetric MD (dB)	Perimetric PSD (dB)	Upper Hemifield	Lower Hemifield	Upper Hemifield	Lower Hemifield	Upper Hemifield	Lower Hemifield
							Total Deviation (dB)	Total Deviation (dB)	PERG Amplitude (μ V)	PERG Amplitude (μ V)	RNFL Thickness (μ m)	RNFL Thickness (μ m)
1	F	80	LE	10	-6.79	6.49	-11.59	-5.53	0.24	0.27	62	58
2	M	63	LE	13	-2.63	2.07	-2.71	-1.71	0.52	0.42	87	83
3	M	78	RE	14	-5.13	9.82	-10.35	-6.06	0.36	0.46	65	53
4	F	48	LE	13	-2.46	2.47	-2.35	-1.94	0.78	0.71	88	83
5	M	75	RE	12	-3.79	2.16	-0.82	-1.35	0.47	0.54	105	110
6	M	53	RE	13	-5.01	2.48	-6.12	-3.24	0.69	0.81	76	68
7	F	51	RE	15	-4.46	2.42	-4.88	-3.06	0.71	0.74	72	80
8	F	74	LE	14	-5.56	2.97	-4.82	-5.47	0.45	0.46	67	82
9	F	39	RE	13	-8.73	14.92	-6.35	-9.71	0.47	0.44	79	64
10	M	49	RE	12	-5.44	6.66	1.12	-4.35	0.35	0.33	101	85

nificant difference, $P < 0.001$). It is noteworthy that none of the eyes showed greater h-PERG values in the affected compared to the unaffected hemifields. Only one eye had identical amplitudes from affected and unaffected hemifields (see Table 1). Median PERG phase did not differ significantly between h-PERGs derived from affected ($-37^\circ \pm 5.7^\circ$) and unaffected ($-36^\circ \pm 5.7^\circ$) hemifields. In diffuse glaucomatous eyes, the median h-PERG amplitudes in the upper

and lower hemifields were 0.47 (range, 0.78–0.24) and 0.46 (range, 0.81–0.27) μ V, respectively. In normal subject eyes, median h-PERG values were 0.83 (range, 1.16–0.63) and 0.78 (range, 1.11–0.64) μ V in the upper and lower hemifields, respectively. No significant differences were found between median h-PERG amplitudes from upper to lower hemifields of diffuse glaucomatous or of normal subject eyes ($P = 0.9$).

The median h-PERG amplitude ratio of (affected/

Table 3. Demographic Characteristics and Hemifield PERG Amplitude Values of Normal Subjects

Normal Subjects #	Sex	Age (yrs)	Eye	Upper Hemifield PERG Amplitude (μ V)	Lower Hemifield PERG Amplitude (μ V)
1	F	34	RE	0.68	1.11
2	F	54	RE	0.81	0.64
3	F	63	LE	0.66	0.76
4	M	82	RE	0.64	0.71
5	M	52	LE	0.85	0.91
6	F	51	LE	0.92	1.02
7	M	38	RE	1.12	0.68
8	F	47	RE	0.91	0.91
9	F	67	LE	0.83	0.72
10	M	79	RE	0.89	0.79
11	M	42	LE	1.16	0.71
12	M	77	LE	0.82	0.92
13	F	71	RE	0.72	0.83
14	M	44	RE	0.93	1.03
15	F	74	LE	0.77	0.87
16	M	81	RE	0.63	0.77
17	F	64	LE	0.83	0.75
18	F	74	RE	0.75	0.65

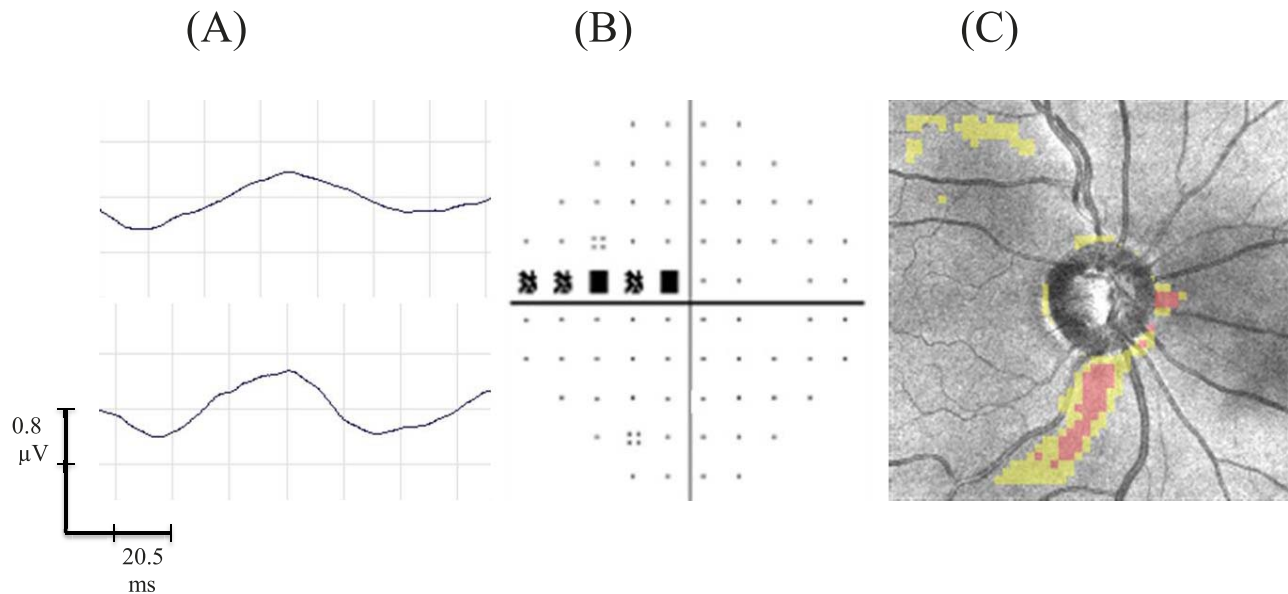


Figure 2. Steady-state h-PERG waveforms and clinical findings obtained in a patient with primary open-angle glaucoma and a superonasal arcuate scotoma (#25 in Table 1). (A) PERG responses from the upper (top) and lower (bottom) hemifields. (B) Automated visual field (total deviation plot from the perimetric 30-2 SITA-standard test). (C) RNFL analysis (thickness deviation map by OCT optic disc cube 200×200). Calibration bars of h-PERG tracings are shown in the bottom left corner.

unaffected) hemifields was 0.67 (range, 0.19–1) in localized glaucoma patients. The ratio of (upper-to-lower) hemifields was 0.97 (range, 0.78–1.24) in diffuse glaucoma patients and 0.92 (range, 0.61–1.65) in normal subjects. The overall change in median amplitude ratios across both groups of glaucoma patients and the normal subjects was highly significant ($P < 0.001$) by the Kruskal-Wallis test. There were significant differences ($P < 0.001$) between the normal and localized glaucoma groups as well as between the localized and diffuse glaucoma groups. However, there was no significant difference in ratio between the normal and diffuse glaucoma groups ($P = 0.9$). No age-related changes in h-PERG amplitude ratio were found in either group ($P = 0.7$).

Median tomographic measurements of RNFL thickness in localized glaucoma patient eyes were 59.5 (range, 90–42) and 89.5 (range, 129–64) μm in the corresponding affected and unaffected hemiretinas, respectively. In strict correspondence with perimetric defects, thickness reductions were found in the superior and inferior RNFL hemifields of 15 and 17 eyes, respectively. The difference in median RNFL thickness in these hemifields was significant ($P < 0.001$). The sign of significant OCT and field abnormality was always consistent with that of h-PERG ratio. Median tomographic measurements of RNFL thickness in diffuse glaucoma patient eyes were 77.5 (range, 105–62) and 81 (range, 110–53) μm

in the upper and lower hemifields, respectively. The difference in median RNFL thickness in these hemifields was not significant.

Figure 2 shows the h-PERG recordings from upper and lower hemifields and the clinical findings (i.e., visual field by Humphrey 30-2 and RNFL analysis by OCT) obtained from the right eye of a patient with localized defect (#25 in Table 1), reflecting a structure–function relationship. In Figure 2A, the upper hemifield PERG (top) was moderately reduced in amplitude compared to the lower hemifield PERG (bottom). In Figure 2B, an arcuate perimetric defect in the superonasal field, close to the fixation point, is displayed. Figure 2C depicts the OCT RNFL deviation map with a bundle defect in the 7 clock-hour sector.

Figure 3 shows box plots of h-PERG amplitude ratios from upper to lower hemifields of normal subjects, affected to unaffected hemifields of localized glaucoma, and upper to lower hemifields of diffuse glaucoma patients. Whereas h-PERG ratios largely overlapped between normals and diffuse glaucoma patients, a clear difference in distribution can be seen between localized glaucoma and the other two groups.

ROC curves were calculated for h-PERG amplitude ratios from affected to unaffected hemifields of localized glaucoma patients and upper to lower hemifields of normal subjects as well as from affected

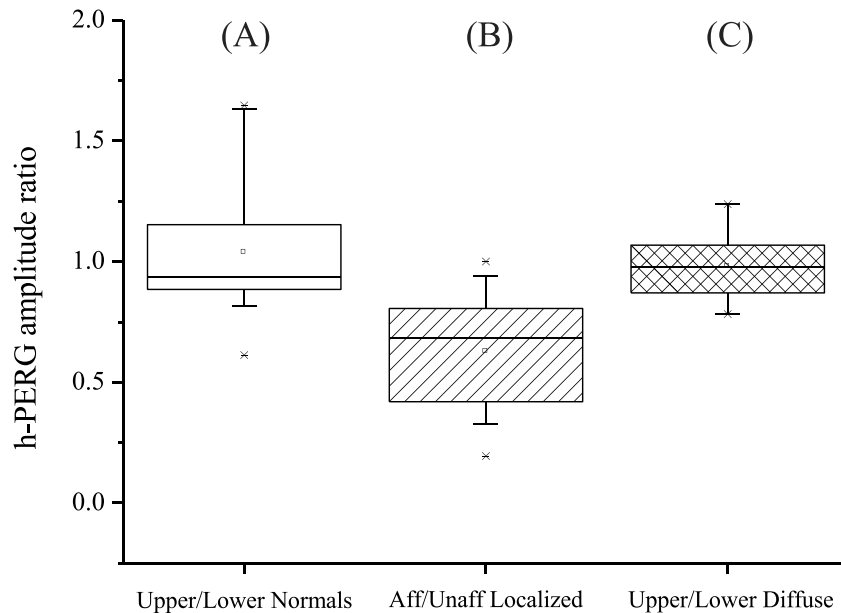


Figure 3. Box plots of h-PERG amplitude ratios calculated from (A) upper and lower hemifields of normal subjects, (B) affected (Aff) and unaffected (Unaff) hemifield of localized glaucoma patients, and (C) upper and lower hemifields of diffuse glaucoma patients. Each box shows 75th, 50th, and 25th percentiles. Error bars show 95th and 5th percentiles. Asterisks show 99th and 1st percentiles.

to unaffected hemifields of localized glaucoma patients and upper to lower hemifields of diffuse glaucoma patients. The AUCs were 0.91 (standard error [SE], 0.04; asymptotic $P < 0.001$; confidence limits, 0.83–0.99) and 0.93 (SE, 0.04; asymptotic $P < 0.001$; confidence limits, 0.85–1), respectively. The cutoff h-PERG ratio value of 0.85 was able to separate the localized glaucoma population from the other two. Indeed, it provided a sensitivity and specificity of 88.9% and 87.5% in discriminating patients with localized glaucoma from normal subjects, and 90% and 87.5% in discriminating localized from diffuse glaucoma patients, respectively. These ROC curves are shown in Figure 4.

Discussion

Our study was designed to determine if, and to what extent, the PERG recorded from a single hemifield may be a reliable indicator of localized defects in glaucoma. The results showed that h-PERG reliably detected focal glaucomatous damage in one hemifield. In addition, the amplitude hemifield ratio was able to discriminate with high accuracy focal glaucomatous eyes from normal subject eyes or from those with diffuse glaucoma.

The amount of amplitude loss of the full-field PERG response, in presence of a focal scotomatous

area (as described in many studies^{10,28,34}), might be underestimated or hidden by the normal response of surrounding healthy areas, and, therefore, undetectable.¹⁴ On the other hand, if the same PERG response loss reflects more a diffuse rather than a localized loss, it may be overestimated and not specific for focal defects (i.e., the PERG detects a “panretinal” damage mechanism and consequently PERG loss may not be congruent with field loss).³⁴ This issue assumes particular relevance when considering that earlier glaucomatous damage often appears in a single hemifield, especially, although not exclusively, in the upper one.³⁵ The current h-PERG protocol is not intended to replace standard diagnostic methodologies (i.e., 30° and 10° central visual fields, spectral-domain OCT RNFL thickness analysis), but rather to be used as a complement of the aforementioned methods to confirm an otherwise uncertain clinical diagnosis of localized glaucoma.

Few studies have investigated PERG recordings from separate hemifields.^{26,27} In 1994, Graham et al.²⁶ evaluated the amplitude of transient and steady-state PERG obtained after stimulation of separate hemifields in normal subjects and glaucoma patients with a one-hemifield defect, and determined an upper-to-lower hemifield PERG ratio. This ratio, for both PERGs, indicated a hemifield asymmetry in either normals and glaucomatous patients. In the latter, however, this asymmetry was more pronounced than

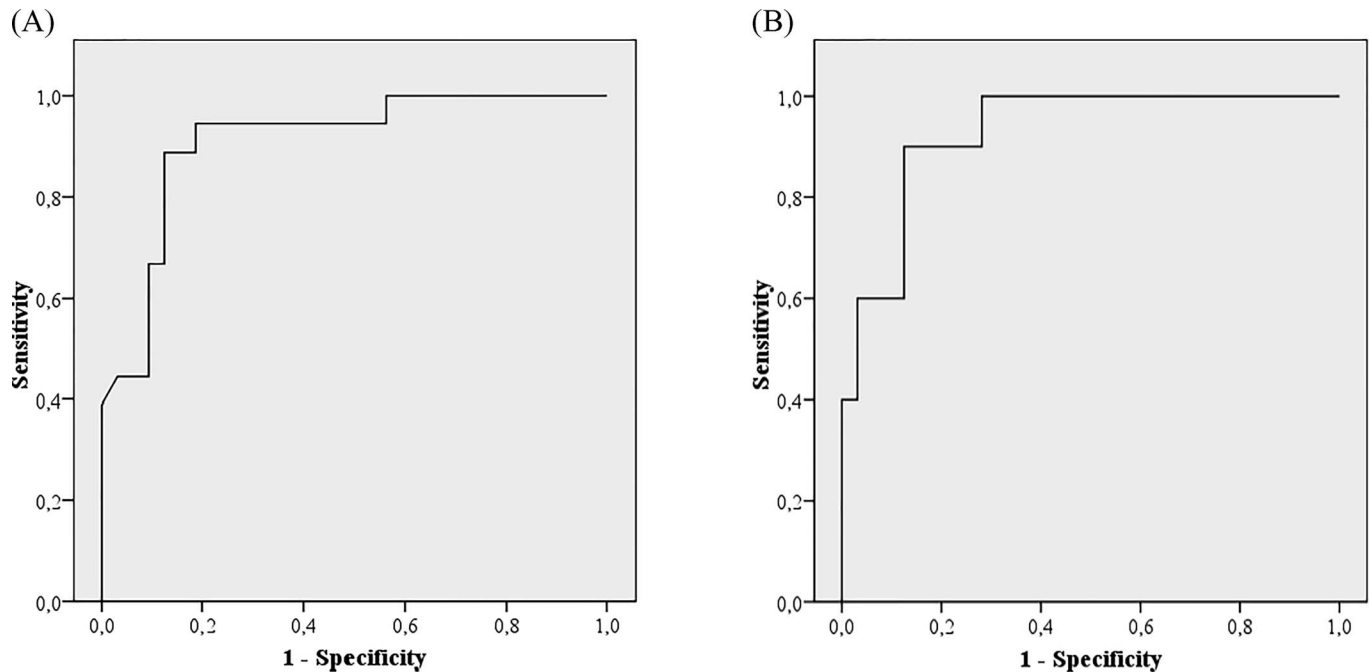


Figure 4. ROC curves calculated for h-PERG amplitude ratios from (A) affected to unaffected hemifields of localized glaucoma patients and upper to lower hemifields of normal subjects ($AUC \pm SE, 0.91 \pm 0.04$) and from (B) affected to unaffected hemifields of localized glaucoma patients and upper to lower hemifields of diffuse glaucoma patients ($AUC \pm SE, 0.93 \pm 0.04$).

in the former, as found in the current study. In addition, the investigators demonstrated that perimetric losses were positively associated with PERG reductions in the same hemifield of glaucoma patients. However, the study included only a limited sample of eight glaucomatous patients.

More recently, Finzi et al.²⁷ investigated transient full-field- and h-PERGs, frequency doubling technology (FDT) visual field and SD-OCT in ocular hypertension patients. They found a statistically significant difference between ocular hypertensives and control subjects when considering N95 amplitudes from upper (4.04 vs. 4.61 μV , respectively) and lower (3.25 vs. 4.43 μV , respectively) hemifields, and not by the full-field recordings. They also found a positive correlation between hemifield N95 amplitudes and Spectralis OCT RNFL thicknesses from corresponding sectors. ROC curves were used to determine and compare the diagnostic sensitivities and specificities of all tests, and showed good diagnostic ability of h-PERG amplitudes, mainly in the lower field, similar to FDT PSD global index and higher than OCT RNFL thickness from the inferior quadrant. Hence, they concluded that h-PERG is a very sensitive test for detecting early glaucomatous damage. In comparison with our study, this report has some methodologic differences. First, the inves-

tigators tested patients with ocular hypertension and no evidence of focal glaucomatous damage at Humphrey perimetry. Second, transient PERG was used, different from our steady-state paradigm. P50 and N95 amplitudes from conventional transient PERG are rather similarly altered in glaucomatous patients, but the steady-state PERG amplitude seems to be more reduced.³⁴ The generators of the transient P50 component may differ from those underlying the N95, wherein the transient P50 is thought to have contributions from outer and middle retina, while the transient N95 and steady-state PERG are thought to be more associated with inner retina/ganglion cells.³⁶ However, Özdamar et al.³⁷ demonstrated a definite relationship between transient and steady-state PERGs, since steady-state PERG resulted from the overlapping of transient PERG waveforms generated at the same reversal rate.

To the best of our knowledge, our study is the first to evaluate the clinical use of steady-state h-PERG in a cohort of glaucomatous patients with well-defined hemifield losses. In this study, steady-state PERGs from separate hemifields were able to differentiate between affected and unaffected hemifields, therefore, confirming the different perimetric conditions. Consequently, ROC curve analyses including data from affected hemiretinas (i.e., evaluating the h-PERG

ratio) demonstrated good diagnostic accuracy of h-PERG, not only comparing localized glaucoma patients versus normal subjects, but also comparing patients with focal versus diffuse field defects. Indeed, the ROC curves allowed us to identify an optimal h-PERG ratio cutoff value of 0.85 for both comparisons (localized vs. normal groups and localized vs. diffuse groups). In the comparison between the localized glaucoma versus normal groups, sensitivity was derived from the proportion of localized glaucoma patients with an abnormal ratio (i.e., ≤ 0.85), whereas specificity was derived from the proportion of normal subjects with a normal ratio (i.e., > 0.85). In the comparison between localized versus diffuse glaucoma groups, sensitivity was derived from the proportion of localized glaucoma patients with an abnormal ratio, whereas specificity was derived from the proportion of diffuse glaucoma patients with a normal ratio. Sensitivity and specificity were nearly 89% and 88%, respectively, for both comparisons. In this respect, similar to Graham's study,²⁶ the patients' h-PERG ratio tended to reflect the selective hemifield loss.

We did not attempt to correlate any quantitative hemifield perimetric difference with corresponding hemifield PERG difference for the following reasons: (1) PERG reflects function of the inner retina, whereas SAP represents integrated activity of the retinal (RGCs) and postretinal (lateral geniculate nucleus and cortex) neural structures, and (2) PERG is a suprathreshold measure of inner retinal function, whereas the perimetric strategy used in this study is based on a threshold measurement.

A potential limitation of our study was that, as already suggested by Graham et al.,²⁶ fixation shifts and stray light effects during stimulation may produce some variation in the response from each hemifield. In addition, similar to full-field PERG and perimetry, h-PERG could be age-related. However, no age-related changes in the h-PERG amplitude ratio were found in our study sample. Other possible limitations in a clinical setting are nonuniform optical media opacities or other concomitant disorders, such as diabetic retinopathy, which may significantly affect the h-PERG ratio. Finally, the effect of the vertical displacement of the fovea relative to the optic disc may represent another confusing factor.³⁸ We believe that these issues should be further addressed in future studies.

Our findings in patients with confirmed focal defect limited to one hemifield indicated a good diagnostic ability of h-PERG, suggesting its use as a

complement to the standard techniques in the diagnosis of localized glaucomatous damage. An application is in cases with an unreliable perimetric response where h-PERG may confirm RNFL findings of focal glaucomatous damage (missing or negative perimetric results with positive OCT findings). Another clinical situation is when RNFL thickness analysis cannot be properly performed; for example, because of congenital optic disc anomalies or lack of patient collaboration (cognitively impaired or non-verbal patients) and unstable fixation. Finally, the use of h-PERG may be of value as an outcome measure for therapeutic approaches aimed at protecting retinal ganglion cells, with the internal control of the healthy unaffected hemifield, similar to that suggested by Mousa et al.³⁹ by using multifocal visual evoked potentials.

Acknowledgments

The authors thank Salvatore Rossi for his help in collecting and analyzing the earliest data.

Supported by Grants (D1, D3) from the Italian Ministry of Research (BF).

Disclosure: **T. Salgarello**, None; **A. Giudiceandrea**, None; **L. Calandriello**, None; **D. Marangoni**, None; **A. Colotto**, None; **A. Caporossi**, None; **B. Falsini**, None

*TS and AG contributed equally to this study.

References

1. Bussel II, Wollstein G, Schuman JS. OCT for glaucoma diagnosis, screening and detection of glaucoma progression. *Br J Ophthalmol*. 2014; 98(suppl 2):ii15–ii19.
2. Larrosa JM, Polo V, Ferreras A, Gil L, Fuentes I, Pablo LE. Predictive value of confocal scanning laser for the onset of visual field loss in glaucoma suspects. *Ophthalmology*. 2012;119:1558–1562.
3. Tatham AJ, Medeiros FA, Zangwill LM, Weinreb RN. Strategies to improve early diagnosis in glaucoma. *Prog Brain Res*. 2015;221:103–133.
4. Quigley HA, Dunkelberger GR, Green WR. Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol*. 1989;107:453–464.
5. Kerrigan-Baumrind LA, Quigley HA, Pease ME, Kerrigan DF, Mitchell RS. Number of ganglion cells in glaucoma eyes compared with threshold

- visual field tests in the same persons. *Invest Ophthalmol Vis Sci*. 2000;41:741–748.
6. Porciatti V, Falsini B, Brunori S, Colotto A, Moretti G. Pattern electroretinogram as a function of spatial frequency in ocular hypertension and early glaucoma. *Doc Ophthalmol*. 1987;65:349–355.
 7. Bach M, Hiss P, Röver J. Check-size specific change of pattern electroretinogram in patients with early open-angle glaucoma. *Doc Ophthalmol*. 1988;69:315–322.
 8. Colotto A, Falsini B, Salgarello T, Iarossi G, Galan ME, Scullica L. Photopic negative response of the human ERG: losses associated with glaucomatous damage. *Invest Ophthalmol Vis Sci*. 2000;41:2205–2211.
 9. Porciatti V, Ventura LM. Normative data for a user-friendly paradigm for pattern electroretinogram recording. *Ophthalmology*. 2004;111:161–168.
 10. Bach M, Poloschek CM. Electrophysiology and glaucoma: current status and future challenges. *Cell Tissue Res*. 2013;353:287–296.
 11. Maffei L, Fiorentini A. Electroretinographic responses to alternating gratings before and after section of the optic nerve. *Science*. 1981;211:953–955.
 12. Fiorentini A, Maffei L, Pirchio M, Spinelli D, Porciatti V. The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway. *Invest Ophthalmol Vis Sci*. 1981;21:490–493.
 13. Pfeiffer N, Tillmon B, Bach M. Predictive value of the pattern electroretinogram in high-risk ocular hypertension. *Invest Ophthalmol Vis Sci*. 1993;34:1710–1715.
 14. Hood DC, Xu L, Thienprasiddhi P, et al. The pattern electroretinogram in glaucoma patients with confirmed visual field deficits. *Invest Ophthalmol Vis Sci*. 2005;46:2411–2418.
 15. Banitt MR, Ventura LM, Feuer WJ, et al. Progressive loss of retinal ganglion cell function precedes structural loss by several years in glaucoma suspects. *Invest Ophthalmol Vis Sci*. 2013;54:2346–2352.
 16. Porciatti V. Electrophysiological assessment of retinal ganglion cell function. *Exp Eye Res*. 2015;141:164–170.
 17. Tuulonen A, Airaksinen PJ. Initial glaucomatous optic disk and retinal nerve fiber layer abnormalities and their progression. *Am J Ophthalmol*. 1991;111:485–490.
 18. Burk RO, Tuulonen A, Airaksinen PJ. Laser scanning tomography of localised nerve fibre layer defects. *Br J Ophthalmol*. 1998;82:1112–1117.
 19. Salgarello T, Colotto A, Valente P, et al. Posterior pole retinal thickness in ocular hypertension and glaucoma: early changes detected by hemispheric asymmetries. *J Glaucoma*. 2005;14:375–383.
 20. Kook MS, Cho HS, Seong M, Choi J. Scanning laser polarimetry using variable corneal compensation in the detection of glaucoma with localized visual field defects. *Ophthalmology*. 2005;112:1970–1978.
 21. Kim NR, Lee ES, Seong GJ, Choi EH, Hong S, Kim CY. Spectral-domain optical coherence tomography for detection of localized retinal nerve fiber layer defects in patients with open-angle glaucoma. *Arch Ophthalmol*. 2010;128:1121–1128.
 22. Na JH, Lee K, Lee JR, Baek S, Yoo SJ, Kook MS. Detection of macular ganglion cell loss in preperimetric glaucoma patients with localized retinal nerve fibre defects by spectral-domain optical coherence tomography. *Clin Experiment Ophthalmol*. 2013;41:870–880.
 23. Asman P, Heijl A. Diffuse visual field loss and glaucoma. *Acta Ophthalmol (Copenh)*. 1994;72:303–308.
 24. Sihota R, Gupta V, Tuli D, Sharma A, Sony P, Srinivasan G. Classifying patterns of localized glaucomatous visual field defects on automated perimetry. *J Glaucoma*. 2007;16:146–152.
 25. Jonas JB, Fernández MC, Stürmer J. Pattern of glaucomatous neuroretinal rim loss. *Ophthalmology*. 1993;100:63–68.
 26. Graham SL, Wong VA, Drance SM, Mikelberg FS. Pattern electroretinograms from hemifields in normal subjects and patients with glaucoma. *Invest Ophthalmol Vis Sci*. 1994;35:3347–3356.
 27. Finzi A, Strobbe E, Tassi F, Fresina M, Cellini M. Hemifield pattern electroretinogram in ocular hypertension: comparison with frequency doubling technology and optical coherence tomography to detect early optic neuropathy. *Clin Ophthalmol*. 2014;8:1929–1936.
 28. Bach M, Sulimma F, Gerling J. Little correlation of the pattern electroretinogram (PERG) and visual field measures in early glaucoma. *Doc Ophthalmol*. 1998;94:253–263.
 29. Keltner JL, Johnson CA, Quigg JM, Cello KE, Kass MA, Gordon MO. Confirmation of visual field abnormalities in the Ocular Hypertension Treatment Study. Ocular Hypertension Treatment Study Group. *Arch Ophthalmol*. 2000;118:1187–1194.

30. Bickler-Bluth M, Trick GL, Kolker AE, Cooper DG. Assessing the utility of reliability indices for automated visual fields. Testing ocular hypertensives. *Ophthalmology*. 1989;96:616–619.
31. Salgarello T, Falsini B, Stifano G, et al. Morpho-functional follow-up of the optic nerve in treated ocular hypertension: disc morphometry and steady-state pattern electroretinogram. *Curr Eye Res*. 2008;33:709–721.
32. Falsini B, Marangoni D, Salgarello T, et al. Structure-function relationship in ocular hypertension and glaucoma: interindividual and interocular analysis by OCT and pattern ERG. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:1153–1162.
33. Yohannan J, Wang J, Brown J, et al. Evidence-based criteria for assessment of visual field reliability. *Ophthalmology*. 2017;124:1612–1620.
34. Bach M, Hoffman MB. Update on the pattern electroretinogram in glaucoma. *Optom Vis Sci*. 2008;85:386–395.
35. Hart WM Jr, Becker B. The onset and evolution of glaucomatous visual field defects. *Ophthalmology*. 1982;89:268–279.
36. Holder GE. Pattern electroretinography (PERG) and an integrated approach to visual pathway diagnosis. *Prog Retin Eye Res*. 2001;20:531–561.
37. Özdamar Ö, Toft-Nielsen J, Bohórquez J, Porciatti V. Relationship between transient and steady-state pattern electroretinograms: theoretical and experimental assessment. *Invest Ophthalmol Vis Sci*. 2014;55:8560–8570.
38. Hood DC, Raza AS, de Moraes CGV, Liebmann JM, Ritch R. Glaucomatous damage of the macula. *Prog Retin Eye Res*. 2013;32:1–21.
39. Mousa MF, Cubbidge RP, Al-Mansouri F, Bener A. The role of hemifield sector analysis in multifocal visual evoked potential objective perimetry in the early detection of glaucomatous visual field defects. *Clin Ophthalmol*. 2013;7:843–858.