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Retinal ganglion cells function measured by the PERG test in patients with ocular hypertension

Funkcja bioelektryczna komórek zwojowych siatkówki mierzona badaniem PERG u pacjentów z nadciśnieniem ocznym

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Summary:

Purpose: To assess the retinal ganglion cells function in patients with ocular hypertension (OHT).

Material and methods: In one hundred eyes of 50 patients with ocular hypertension [mean age: 48 ± 13 years, intraocular pressure mean: 26 ± 3.0 mmHg; Humphrey Field Analyzer (HFA) 24-2 W-W mean deviation (MD) > -2 decibels (dB), normal optic nerve fiber layer results in scanning laser polarimetry (GDX)], PERG recordings were performed according to the modified methodology described by Parisi V et al. (Ophthalmology 2006; 113: 216-228). Main parameters measured in the PERG test were as follows: amplitude of the P50 and N95 waves, AN95/AP50 ratio as well as implicit time of P50 wave. Pattern Electroretinogram P50 implicit times were considered delayed when exceeding the limit of mean values plus 2 standard deviations (SDs) from controls. PERG amplitudes of P50, N95 waves and AN95/AP50 ratio were considered reduced when they were below the mean values minus 2 SDs from controls. Control group consisted of age, sex, and refractive error matched one hundred eyes of 50 healthy subjects with intraocular pressure mean equal to 16 ± 4.0 mmHg.

Results: In the OHT eyes, significant reductions of AN95-wave ($p < 0.0002$) and AN95/AP50 ratio ($p < 0.002$) were obtained in comparison to controls. The PERG test revealed the abnormalities mostly in AN95/AP50 ratio (32/100 of OHT eyes – 32%). Other less frequent abnormalities were observed in amplitude of N95-wave (10/100 of OHT eyes – 10%) and in amplitude of P50-wave (6/100 of OHT eyes – 6%). The implicit times of P50 and N95-waves were within normal limits.

Conclusions: In the eyes of the OHT patients, retinal ganglion cells dysfunction is present and can be detected by the PERG test. Longer follow-up is necessary to show predictable value of the PERG examination in separation of the OHT patients with a high risk of glaucoma development.

Key words: ganglion cells function, PERG, ocular hypertension (OHT).

Streszczenie:

Cel: celem pracy jest ocena funkcji bioelektrycznej komórek zwojowych siatkówki u pacjentów z nadciśnieniem ocznym (OHT).

Pacjenci i metody: 100 oczu 50 pacjentów z OHT [średni wiek: 48 ± 13 lat, średnie ciśnienie śródgałkowe 26 ± 3,0 mHg; perymetria statyczna PS-24-2 (W-W), „full threshold", MD > -2,0 dB, prawidłowa warstwa włókien nerwowych siatkówki w badaniu skaningowej polarymetrii laserowej GDx] poddano badaniu PERG wg zmodyfikowanej metodyki, którą opisali Parisi i wsp. (Ophthalmology 2006; 113: 216-228). Oceniano parametry: amplitudę fal P50 i N95, stosunek amplitud fal N95 do P50 (AN95/AP50), a także czas kulminacji fali P50. Czas kulminacji fali P50 uznawano za nieprawidłowy, gdy przekraczał zakres dwóch odchyleń standardowych (SD) powyżej średniej z grupy kontrolnej. Amplitudy fal P50, N95 i stosunek amplitud fal N95 do P50 uważano za zredukowane, gdy pozostawały poniżej zakresu dwóch odchyleń standardowych od średniej z grupy porównawczej. Grupę porównawczą stanowiło 100 oczu 50 zdrowych osób ze średnim ciśnieniem śródgałkowym 16 ± 4,0 mmHg, zgodnych pod względem wieku, płci i wady refrakcji.

Wyniki: w oczach z OHT stwierdzono statystycznie istotną redukcję amplitudy fali N95 ($p<0,0002$) i stosunku amplitud fal N95/P50 ($p<0,002$) w porównaniu do grupy kontrolnej. W badaniu PERG najczęstszą nieprawidłowością był obniżony stosunek AN95/AP50 (32/100 oczu z OHT – 32%). Z mniejszą częstotliwością występowało obniżenie amplitudy fali N95 (10/100 oczu z OHT – 10%) oraz obniżenie amplitudy fali P50 (6/100 oczu z OHT – 6%). Czas kulminacji fal P50 i N95 pozostawał w granicach normy.

Wnioski: u pacjentów z OHT stwierdzono zaburzenie funkcji bioelektrycznej komórek zwojowych manifestujące się w badaniu PERG. Odpowiedź na pytanie, czy badanie PERG będzie przydatne w procesie wyodrębniania grupy wysokiego ryzyka konwersji OHT do jaskry, wymaga dalszych, długoterminowych badań.

Słowa kluczowe: funkcja bioelektryczna komórek zwojowych, PERG, nadciśnienie oczne (OHT).

Introduction

Ocular hypertension (OHT) is defined as increased intraocular pressure without glaucomatous losses in the visual field or changes in the optic nerve. Increased pressure is a key factor in the development of glaucoma, which is characterized by chronic damage to the ganglion cells of the retina. Up to 25-35%

ISSN 0023-2157 Index 362646

of the ganglion cells must be damaged to record a loss of vision, using standard static perimetry (1).

The facts outlined above suggest that all patients with OHT should receive medication to lower the intraocular pressure preventively. The results of previous studies carried out show that only 1% of patients with intraocular pressure equal to 25 mmHg expand each year glaucoma. In prospective research, frequency of glaucoma varies from 0.4% to 17.4% (2-5). Thus, in the majority of patients with OHT never gets to glaucoma. To avoid unnecessary treatment of many patients with the OHT, it is indicated to identify the patients who develop full-symptom glaucoma in the future.

Pattern Electroretinogram (PERG) is the indicator of the function of ganglion cells (6,7). Invalid PERG exists in many patients with early glaucoma and OHT (8-13) which suggests that the damage to the ganglion cells is an instance of the changes in the visual field. In glaucoma, the PERG test may be used in early diagnosis (13), monitoring the progression (9), monitoring the favorable impact on ganglion cells drugs reducing the intraocular pressure (14). In the early glaucoma PERG may be invalid, despite the absence of changes in the morphology of the optic nerve disc, measured by means of optical coherent tomography (OCT) (15).

In the patients with OHT and correct result of the visual field measurements, reduced amplitude of the PERG was observed (16-19). The results of the few authors show that invalid PERG in the OHT may be an indicator of full-symptom glaucoma development (16,18,20).

Therefore the aim of the present work was to assess the own material, to determine whether in patients with increased intraocular pressure there is a disorder of ganglion cell function demonstrated by incorrect PERG, and if the PERG test has potential use in separation of the group with a particular risk of glaucoma development.

Material and methods

The research material was 100 eyes from 50 patients with OHT mean of age 48 $\pm$ 12 years. The criteria for inclusion in studies of patients with OHT were the following:

- the intraocular pressure measured with Goldmann applanation tonometer (including pachymetry), $\geq$ 22 mmHg and $>$ 29 mmHg without treatment (average of the two measurements at 9:00 and 16:00 every day, performed during 6 days), confirmed by 2 independent investigators;
- static perimetry – 24-2 (white on white), „full threshold", MD $>$ -2.0 dB, PSD $<$ +2.0 dB, fixation looses, frequency of false-positive and negative $<$ 20% (Humphrey Perimeter model 750);
- best corrected far visual acuity of 20/20 (LogMAR scale);
- refraction errors of no more than $\pm$ 3 D;
- correct optic nerve disc analyzed on the basis of the eye images, performed with the Zeiss Fundus camera model FF 450 (excluded: the local and polar fiber layer loss of the optic nerve (notch, peripapillary splinter hemorrhages), inter-ocular asymmetry c/d $<$ 0.2;
- open angle;
- the absence of diseases of the eye and system of known effects on the functions of the retina and optic nerve;
- pupil diameter $\geq$ 3 mm.

In order to evaluate the thickness of the optic nerve fiber layer in 15 degrees area around the disc, the measurements were made with the GDx-VCC Nerve fiber Analyzer (Carl Zeiss Meditec), version 5.5.1. The following parameters were evaluated: TSNIT Average, Superior average, Inferior average, TSNIT Standard Deviation, Inter-eye Symmetry, NFI. In the tested eyes of patients with the OHT, GDX test results were within the normal limits (TSNIT Average 68.3 $\pm$ 12; Superior Average 81.1 $\pm$ 9; Inferior Average 86.2 $\pm$ 10; TSNIT Std. Dev. 28.1 $\pm$ 6; Inter-Eye Symmetry 0.95 $\pm$ 0.2; NFI 11 $\pm$ 6), in comparison to the normative data included in the GDx system.

Control group was 100 eyes from 50 of healthy, matched to the test group as to gender, age and errors of refraction.

The subjects from the test and control groups expressed their written consent to participation in research. The project obtained the approval of the Bio-ethics Commission of the Pomeranian Medical University (No. BN-001/103/06).

The transient PERG test was performed according to the modified methodology proposed by Parisi et al. (21). Modification of the test applied was because in their work the authors obtained a high frequency of abnormalities in the PERG examination in patients with OHT (85% of patients), which pointed to the high sensitivity of this methodology (better than that recommended by the ISCEV) in detection of the retinal ganglion cells dysfunction.

Stimulation

Monocular (first RE, then LE), the angular size of the test field (screen) = 17°, a single check = 0° 15' (64 checks in the vertical axis), distance from the eye of a patient to the screen = 1 m, contrast = 80%, screen luminance = 110 nt, mode of modulation: contrast reversal, frequency of stimulation = 2.34 rev/sec (1.17 Hz).

Electrodes

Active electrode – DTL, reference electrode – surface, gold-cup with conducting gel, fixed at outer cantus of the eye, ground electrode – of the same type, located on the ear-lobe opposite to the tested eye.

Recording, analysis and measurement of waveforms

Recording bandwidth: 1-50 Hz, time of analysis – 250 ms, averages – 200 waveforms; two consecutive recordings obtained for the same eye were then off-line averaged and subjected to further analysis; amplitudes as well as implicit times of the P50-wave and N95-wave, and the amplitude ratio N95/P50 were measured; signal-to-noise ratio was assessed for all recordings and the test was considered to be technically performed correctly when the signal-to-noise ratio was $>$ 2.

Clinical protocol

Position of the patient was comfortable, with a chin-rest, the pupils were not dilated, central fixation was used; to prevent frequent blinking, breaks in the examination were introduced; optimum refraction correction for a distance of 1 m was provided.

A sample normal PERG signal and noise are shown on Figure 1.

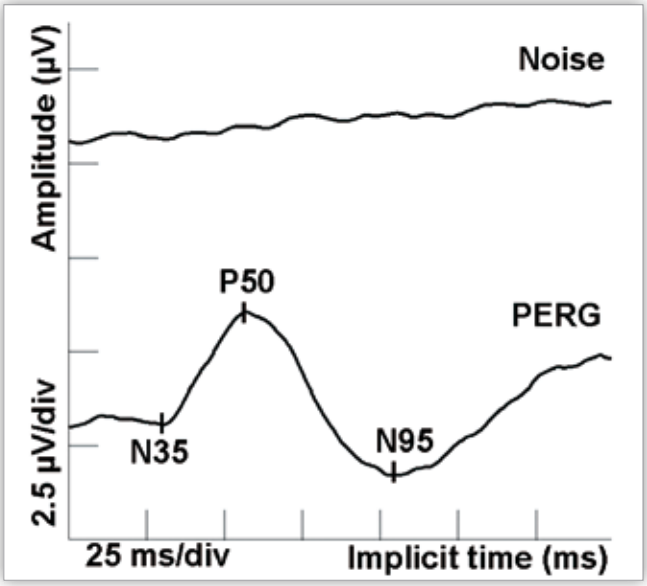

Fig. 1. An example of normal PERG and noise record.
Ryc. 1. Prawidłowy zapis PERG oraz rejestracja szumu.

The PERG test results from the group of OHT patients were compared with the results obtained in the control group.

Statistical analysis

Compatibility of the experimental data with the normal distribution was analyzed using the Shapiro-Wilk test. For the features compatible with the normal distribution, arithmetic mean (X) and standard deviation (SD) were calculated. For the features with distribution that was incompatible with the normal distribution, the range of variation and the median were given. Comparison of parameters of the test and control groups was made according to the commonly used rules, with parametric or non-parametric test of significance for two independent samples. For features compatible with normal distribution, a Z-test was used. For features incompatible with normal distribution, even in only one of the tests, comparisons were made using the non-parametric Mann-Whitney U-test of significance. Acceptable probability of error of the first kind (significance level of the test), was $p = 0.05$. For individual patient, electrophysiological parameters were treated as normal, if they were between $X \pm 2$ SD for the normal distribution, and between 2.5 and 97.5 percentile for the statistical distribution different from the normal. In accordance with the methodology adopted in most of the work in electrophysiology of vision, test sample was determined on the basis of the number of eyes, not the number of patients.

Results

In the group with the OHT, the average intraocular pressure was significantly increased as compared to the control group of healthy subjects (26.0 ± 3.0 mmHg versus 16.0 ± 4 mmHg, $p < 0.02$).

Table I presents a statistical analysis of the PERG test parameters (AP50, ITP50, AN95, ITN95, AN95/AP50 ratio) in the group with the OHT and control ($n = 100$ eyes).

In the eyes of patients with the OHT, statistically significant reduction of the mean amplitude of the N95-wave ($p < 0.0002$) was observed, as well as decreased average ratio of the amplitudes N95 to P50 ($p < 0.002$) compared with control group of healthy eyes. Invalid AN95/AP50 ratio was a consequence of reduction of the AN95. The average amplitude of the P50-wave and implicit times of the N95 and P50 waves did not differ significantly in the test (OHT) and control group.

Parameter/ Parametr	Group/ Grupa	M	SD	Min	Med	Max	N	p
IT P50 (ms)	C	58.2	3.8	51	**57**	70	-	0.239
	OHT	57.7	4.5	49	**57**	86	-	
A P50 (μV)	C	**2.2**	0.68	0.77	2.18	3.7	+	0.194
	OHT	2.12	0.84	0.78	**2.05**	3.64	-	
A N95 (μV)	C	**4.15**	**1.17**	1.29	4.25	7.2	+	**0.0002***
	OHT	**3.47**	**1.39**	0.67	3.3	6.81	+	
AN95 / AP50	C	1.9	0.25	1.42	**1.87**	2.34	-	**0.002****
	OHT	1.64	0.72	0.72	**1.64**	2.73	-	

Tab. I. Statistical analysis of the PERG test parameters (AP50, ITP50, AN95, ITN95, AN95/AP50 ratio), in the group of patients with the OHT and control group ($n = 100$ eyes).
Tab. I. Analiza statystyczna parametrów PERG (AP50, ITP50, AN95, ITN95, stosunek AN95/AP50) w grupie pacjentów z OHT i grupie kontrolnej ($n = 100$ oczu).
A – amplitude/ amplituda; IT – implicit time/ czas kulminacji
C – control group/ grupa kontrolna; OHT – OHT group/ grupa z OHT
M – arithmetic mean/ średnia arytmetyczna; SD – standard deviation/ odchylenie standardowe
Min – minimum value/ minimum; Med – medium value/ mediana; Max – maximum value/ maximum
N – normal distribution/ rozkład normalny; p – significance level/ poziom istotności * – Z-test; ** – Mann-Whitney U-test

Table II shows the range of normal values for the PERG test parameters analyzed, the observed abnormalities and their frequency in the group of patients with OHT.

Parameter/ Parametr	Normal range/ Zakres normy	Frequency of abnormalities (number of eyes)/ Częstość nieprawidłowowości (liczba oczu)
AP50 (μV)	0.84-3.56	↓ A P50 (6/100) **6%**
AN95 (μV)	1.81-6.49	↓ A N95 (10/100) **10%**
ratio AN95/ AP50	1.43-2.33	↓ A N95/ A P50 (32/100) **32%**

Tab. II. Range of normal values for the PERG test parameters analyzed, the observed abnormalities and their frequency in the group of patients with OHT.
Tab. II. Zakres wartości prawidłowych analizowanych parametrów PERG, obserwowane nieprawidłowości i częstość ich występowania w grupie pacjentów z OHT.
A – amplitude/ amplituda

When the PERG test parameters were examined in individual patients with the OHT, reduction of AP50 in 6/100 eyes (6%) and reduction of AN95 in 10/100 eyes (10%) was found compared to the range of normal values obtained on the basis of averages derived from analysis of the results in the control group. The most common abnormality was the reduced AN95/ AP50 ratio (32/100 eyes – 32%).

ISSN 0023-2157 Index 362646

Figure 2 shows the abnormal PERG recording (decreased ratio AN95/AP50) in a patient with OHT in comparison to normal PERG from the control group.

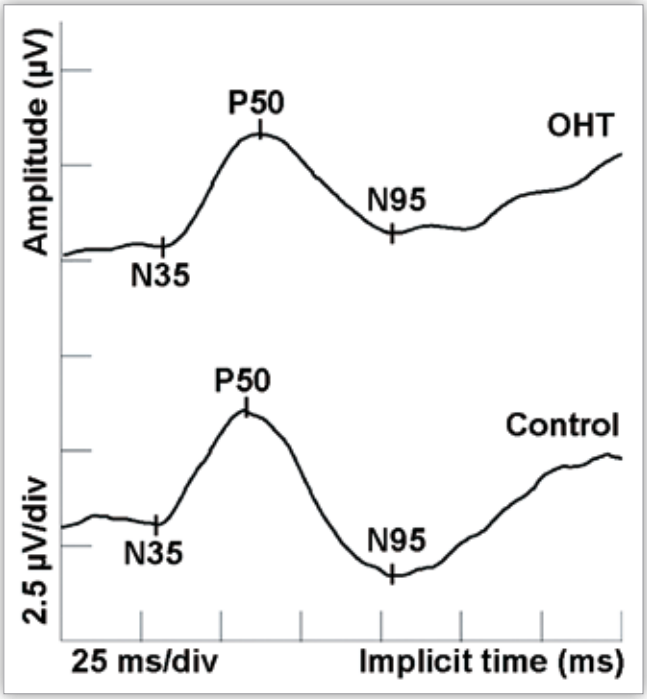

Fig. 2. Abnormal PERG record (decreased ratio AN95/AP50), in a patient with OHT in comparison to normal PERG from the control group.

Ryc. 2. Nieprawidłowy zapis PERG (obniżony stosunek amplitud fal N95/P50) u pacjenta z OHT w porównaniu z zapisem prawidłowym PERG u osób w grupie kontrolnej.

Discussion

It is considered that the reduction of the PERG signal amplitude is the result of the loss of ganglion cells of the retina, their incorrect function, or both. Increased implicit times of the wave is caused by the dysfunction of ganglion cell dendrites or delay of axonal transport (22). In the present work changes in PERG manifested itself as a disturbance of the AN95/AP50 ratio and reduction of the amplitude of waves, with the co-existing normal thickness of optic nerve fibers obtained in the GDX Test (Table I and II). Considering the current results and the results of other studies (23), in which it was shown that in OHT the PERG amplitude reduction existed in parallel with normal thickness of optic nerve fibers measured using OCT, we believe that in the eyes of the OHT patients analyzed by us the dysfunction of the retinal ganglion cells exists.

When the PERG results of a single patient with the OHT were assessed, over 1/3 (32%) of the eyes demonstrated dysfunction of the ganglion cells by reduced AN95/AP50 ratio, as a consequence of the relative reduction of amplitude of the N95-wave. Much less frequently, *i.e.* in 10% of the examined eyes, the reduction of amplitude of the N95-wave was found, and only in 6% – reduction of amplitude of the P50-wave. The most common occurrence of abnormal AP50/AN95 ratio in the PERG test (Tab. II), is the logical consequence of small functional disturbances of the retinal ganglion cells which can be expected in certain patients with the OHT (Fig. 2).

An absolute reduction of amplitude of the PERG N95-wave is most often in individuals with recognized, advanced glaucomatous neuropathy following mainly a significant loss of ganglion cells.

Abnormalities in PERG examination in patients with the OHT were observed in the past by other authors (13,16,19,24) and were of the incidence of 41-73%, in the work of Parisi and coworkers (21) of 69%, in our study – of 32%. Differences of frequency of abnormal PERG in the OHT may result from the lack of uniformity of research methodologies as well as using various criteria for the selection of patients for tests (e.g. demographic differences, average deviation in perimetry, visual appearance of the optic nerve disc or intraocular pressure).

Till now, only 2 long-term studies were realized evaluating whether the PERG test can identify the eyes with the OHT which in the future develop glaucoma. In one of them (16), the PERG test was performed in 29 eyes of 18 patients, and PERG recordings were found invalid in 12 eyes. In 5 of them, in the period 1-3 years, full-symptom glaucoma has developed. It is important that glaucoma has not developed in any of the patients with originally normal PERG recordings. In another study (9) Bach and colleagues presented 8 years (in average) observations of 95 eyes from 54 patients with OHT and intraocular pressure $>$ 25 mmHg. During this period, in 8 eyes of 5 patients developed glaucoma (in 8% of the tested eyes with OHT), while the PERG allowed the estimation of stability or progression to glaucoma at least 1 year before the conversion. These test results indicate clearly that the examination of PERG may have diagnostic value in extracting the eyes with the OHT with high risk of developing glaucoma.

In the present study, being a first stage of our research aimed at determining the clinical value of the PERG test in the OHT, we showed the occurrence of retinal ganglion cells dysfunction. It was demonstrated not only in comparisons between groups, but also in about 30% of analyzed eyes with the OHT. The answer to the question of whether an abnormal PERG test result in OHT will indicate the patients at risk of glaucoma development requires further long-term research. Separation of such patients may have critical importance for them. The inclusion of the appropriate anti-glaucoma treatment at the early stage of functional damage to ganglion cells and not only after detecting the structural changes can prevent severe loss in the visual field and significantly slow down the progression of this disease.

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The study was originally received 08.02.2011 (1274)/
Praca wpłynęła do Redakcji 08.02.2011 (1274)/
Accepted for publication 30.03.2011/
Zakwalifikowano do druku 30.03.2011 r.

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ISSN 0023-2157 Index 362646