Effect of bilateral asymptomatic internal carotid artery stenosis on electrophysiological function of the retina and optic nerve

Wpływ bezobjawowego obustronnego zwężenia tętnicy szyjnej wewnętrznej na funkcję bioelektryczną siatkówki i nerwu wzrokowego

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Abstract:
Aim: This study was designed to assess the bioelectrical function of the retina and optic nerve in patients with bilateral, asymptomatic, yet hemodynamically significant internal carotid artery stenosis.

Material and methods: Twenty-six eyes of 13 subjects with bilateral internal carotid artery stenosis and 34 controls were analyzed. Pattern electroretinogram, full-field electroretinogram and pattern visual evoked potentials, as well as routine ophthalmic examination were performed.

Results: The pattern electroretinogram P50 and N95 wave amplitudes were significantly reduced in patients (p = .0001 and p < .0001 respectively) as compared to controls. The full-field electroretinogram rod b-wave, rod-cone a-wave and b-wave amplitudes were reduced (p = .03, p = .02 and p = .03, respectively) and the implicit times of rod b-wave and rod-cone b-wave were significantly prolonged (p = .01 and p = .02, respectively), whereas the oscillatory potential wave index was reduced (p = .009) in patients as compared to controls. When analyzing cone responses, 30-Hz flicker wave amplitudes were reduced (p = .01) and cone a-wave and b-wave single flash implicit times were significantly prolonged (p = .0009 and p = .02, respectively) in patients as compared to controls.

Conclusions: Our study demonstrated that retinal bioelectrical function is negatively affected by internal carotid artery stenosis despite the absence of objective clinical signs and symptoms of ocular ischemia.

Key words: internal carotid artery stenosis, electroretinogram, retinal ischemia.

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Introduction
Clinical associations between retinal ischemia and internal carotid artery stenosis (ICAS) are well established. The ophthalmic manifestations of carotid insufficiency vary according to the ICAS extent and duration, including transient episodes of monocular blindness (referred to as amaurosis fugax), venous stasis retinopathy, ocular ischemic syndrome with iris neovascular beaded vessels. The most common ocular symptom, which occurs in 30–40% of patients with atherosclerotic carotid occlusive disease, is ipsilateral transient visual loss (2). Over 90% of patients with transient monocular visual loss have abnormal carotid angiograms (3). Furthermore, affected patients are also at risk of developing ocular/orbital pain, aqueous flare, and cataract. Fluorescein angiography demonstrated delayed and patchy choroidal filling and diffuse leakage from the retinal vessels and the optic nerve head (4). Interestingly, ocular signs and symptoms may represent the first manifestation of carotid stenosis (2).

In general, retinal assessment of patients with ICAS is carried out upon onset of clinical ocular symptoms. However, we hypothesize that prior to the onset of ocular ICAS symptoms, the choroid and retina may already be affected by ischemia which alters electrophysiological retinal function at a subclinical level. Such dysfunction may even represent its unique detectable manifestation. The incidence of abnormal retinal function in asymptomatic ICAS secondary to atherosclerotic carotid artery disease has previously been investigated using a full-field ERG and multifocal ERG (mERG) to a limited extent (5, 6). As ERG and mERG originate from preganglionic elements (7), these electrophysiological tests do not enable the functional evaluation of the innermost retinal layers, particularly in the macular region. In contrast, the function of ganglion cells and their fibers may be specifically assessed by pattern electroretinogram (PERG) and pattern visual evoked potentials (PVEP) (8). Nevertheless, PERG and PVEP results in patients with significant ICAS are currently unknown.

Therefore, the present study was designed to assess retinal and optic nerve bioelectrical function using ERG, combined with PERG and PVEP in both eyes of patients with asymptomatic bilateral hemodynamically significant ICAS without objective signs and symptoms of ocular ischemia.

Material and methods

Study group characteristics

Twenty-six eyes of 13 subjects with bilateral hemodynamically significant ICAS (i.e., ≥ 70% of ICA diameter reduction in neurologically asymptomatic patients, defined as subjects without chronic or transient ischemic symptoms typical of carotid territory or transient ipsilateral blindness in the previous six months prior to study enrolment) were analyzed. The subjects were randomly selected from a group of patients of the Department of Vascular Surgery at the Pomeranian Medical University in Szczecin, Poland, eligible for carotid endarterectomy (CEA). Thirty-four age- and sex-matched volunteers without signs of carotid stenosis or exceptionally with ICAS at a grade lower than 40% were enrolled as a control group. ICAS grade was determined in each subject using carotid color Doppler ultrasonography (CDU) using a Voluson 730 PRO, GE Medical Systems device (Milwaukee, WI, USA). Patients with ocular ischemic syndrome or evidence of chronic disease in the fellow eye or a systemic disease that potentially affects the retina and optic nerve, i.e., glaucoma, intraocular inflammatory diseases, retinopathy, recent (within 3 months) ocular surgery, advanced cataract, collagen-related or neoplastic disease, or a history of previous contralateral/unilateral CEA or ICAS stenting were excluded from the study. All enrolled subjects underwent a complete ophthalmic examination of both eyes, i.e., distant best-corrected visual acuity (BCVA), intraocular pressure (IOP) measurements, and anterior segment and dilated ophthalmoscopy fundus examinations using slit-lamp biomicroscopy. Ophthalmic inclusion criteria included a best-corrected distance visual acuity (VA) > .8 (Snellen chart) and normal findings in a routine eye examination.

Medical history, current drug use and smoking status were ascertained along with laboratory data, pathology tests and other information, with particular focus on cardiovascular conditions and pre-existing hypertension. Cumulative pack-years were calculated using the reported average number of cigarettes smoked per day and the number of years of smoking.

The study adhered to the tenets of the Declaration of Helsinki, and approval was obtained from the Local Research Ethics Committee. Moreover, each patient provided written informed consent for their participation.

Electrophysiology

Full-field electroretinograms (UTAS-E 2000 system, LKC Technologies, Inc.), pattern electroretinograms and pattern visual evoked potentials (RetiPort System, Roland Consult Instr.) were recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) protocols. For full-field ERG, recordings were performed binocularly with the use of bipolar Burian-Allen electrodes (Hansen Ophthalmic Development Lab, Iowa, USA) and a ground–clip gold electrode (Natus Europe GmbH, Planegg, Germany) attached to the earlobe. Prior to recording, the cornea was anesthetized (Alcaine; Alcon), and the pupils were dilated with 1% tropicamide eye drops. The recording system parameters were as follows: amplifier sensitivity: 10–20–50 µV/div; filters: 0.3–500 Hz (for OP extraction: 75–500 Hz), notch filters: off, time base: 5 ms/div, and artifact reject threshold: 0 µV. A 30-min dark adaptation preceded the rod and mixed cone-rod recordings; a 10-min period of light adaptation preceded the photopic cone and flicker recordings. The rod ERG recordings were obtained with single dim white flashes of 1.6 cd·s·m−2 attenuated by a 24-dB neutral filter. The mixed rod-cone ERGs were elicited with flashes of white light of 1.6 cd·s·m−2. The cone ERGs were elicited with white flashes of the same intensity. In the flicker recordings, the frequency of stimulation was 30 Hz (the same conditions of adaptation), and ten sweeps were averaged. The oscillatory potentials in the scotopic state were obtained with 1.6 cd·s·m−2 white flashes. The overall wave index of the OP amplitudes (01 + 02 + 03) was measured.

For PERG, monocular stimulation was used, in combination with an appropriate refractive error correction in relation to the eye-screen distance. The examination was interrupted when frequent blinking or fixation losses were identified (the pa-
tient was monitored with a TV camera). The pupils were not
dilated, and central fixation was used. The PERG stimulation pa-
rameters were as follows: a 21° CRT monitor with a frame rate
equal to 70 fps was used; dimension of the stimulus field was
1502° (the mean of the width and the height of the screen),
with the aspect ratio between the width and the height (screen
proportion H/V) equal to 4:3; black and white reversing checker-
board was presented to the patient, with a check size equal to
0048; luminance for white elements was equal to 120 cd/m²,
mean luminance of the stimulus screen: 62 cd/m², with Mi-
chelson contrast set to 97%; temporal frequency was equal
to 4.0 rps (2.0 Hz). The electrodes were as follows: a ground
(gold disk) electrode (Roland Consult, Brandenburg, Germany)
was placed on the forehead (Fpz), a thread Dawson-Trick-Litz-
kov (DTL) electrode (Diagnosys LLC, MA, USA) was used as
active, a gold disk placed at the ipsilateral outer canthus was
used as a reference. The recording system parameters were as
follows: amplifier sensitivity 20 µV/div, filters: 1–100 Hz, arti-
fact reject threshold 95% (for the amplifier range ± 100 µV),
notch filters were off, averaging: 200 sweeps, and sweep time:
250 ms (time base: 25 ms/div). Two consecutive waveforms
were recorded; they were subsequently averaged and analyzed
off-line.
For pattern visual evoked potentials, monocular stimulation
was used without pupil dilation and refraction correction was
applied with respect to the eye-screen distance. Central fixation
was used. Patient was monitored with a TV camera, and inter-
ruptions of the tests were introduced when fixation loose or fre-
cquent blinking was observed. 21” CRT monitor with a frame
rate equal to 70 fps was used for pattern stimulation; dimension
of the stimulus field was 170.0 mm (the height of the screen),
with aspect ratio between the width and the height (screen pro-
portion H/V) equal to 4:3; black and white reversing checker-
board was presented to the patient, with a check size equal to
0048; luminance for white elements was equal to 120 cd/m²,
mean luminance of the stimulus screen: 62 cd/m², with Mi-
chelson contrast set to 97%; temporal frequency was equal
to 4.0 rps (2.0 Hz). The electrodes were as follows: a ground
(gold disk) electrode was placed on the forehead at Fpz. Before
recordings, inter-electrode impedance was measured; values < 5 kΩ
were accepted. Parameters of the recording channel were as follows: amplifiers range ±100 µV/div, filter
center frequency bandwidth 1–100 Hz. Analysis period (sweep time)
was equal to 300 ms. Artifact reject threshold was set to 95%,
and 100 sweeps were averaged. For each eye and each check
size two consecutive PVEP waveforms were recorded and off-
line averaged for further analysis.

**Statistical analysis**

Parameters were compared between independent gro-
ups with the Mann-Whitney test for quantitative variables
and the Fisher’s exact test for qualitative variables. The strength
of the association between the quantitative variables was me-
asured with Spearman rank correlation coefficient (Rs). A
p < 0.05 was considered statistically significant.

**Results**

Twenty-six eyes of 13 patients (4 men, 9 women; mean age
of 65.2 ± 6.8 years) with significant bilateral ICAS were en-
rolled. The mean extent of internal carotid stenosis was 81.9
± 6.6%. Sixty-eight eyes of 34 controls (23 women, 21 men;
mean age of 62.5 ± 4.0 years) were studied, as well.

Table I provides the values of PERG and PVEP measu-
rements obtained for all eyes. The PERG P50 and N95 wave
amplitudes were significantly reduced in ICAS group compared
with the controls. When analyzing the VEP parameters, there
was no significant difference in P100 wave amplitudes between
patients and controls, however the P100 wave latency was si-
gnificantly prolonged in the study group.

The values of the full-field ERG amplitudes and peak times
for all groups are summarized in Table II. The rod b-wave am-
plitudes were significantly decreased (p = .03) and the implicit
times were significantly prolonged (p = .01) in the ICAS group
compared with the controls. Similarly, in the ICAS group, the rod
a-wave amplitudes were significantly decreased (p = .002)
compared with the controls. Furthermore, in the ICAS eyes,
we identified a significant decrease in the rod-cone b-wave am-
plitudes (p = .03) and a delay in the b-wave peak times (p
= .02). When the OP wave index was analyzed, a significant

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<th>Amplitude/ Amplituda (µV)</th>
<th>Peak time/ Latencja (ms)</th>
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<tr>
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<td>study group/ grupa badana</td>
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<td>PERG</td>
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<td>P50</td>
<td>2.24 ± 1.51</td>
<td>3.65 ± 1.51</td>
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<td>N95</td>
<td>3.36 ± 2.27</td>
<td>5.62 ± 2.24</td>
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<td>PVEP</td>
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<td>P100 (1°4')</td>
<td>8.92 ± 4.77</td>
<td>7.96 ± 3.47</td>
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<td>P100 (0°8')</td>
<td>10.96 ± 6.83</td>
<td>9.15 ± 5.13</td>
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*pStatistically significant values were given in bold/ Wartości istotne statystycznie wyraźnie wyraźne
Tab. I. Mean values (± standard deviation) of PERG and VER amplitudes and peak times in study and control groups.
Tab. I. Średnie wartości (± odchylenie standardowe) amplitud i latencji PERG i VER w badanych grupach.
Effect of bilateral asymptomatic internal carotid artery stenosis on electrophysiological function of the retina and optic nerve

| Table II. Mean values (± standard deviation) of full field electroretinogram amplitudes and peak times in study and control groups. |
|---|---|---|---|---|---|---|---|---|---|
| **Amplitude/ Amplitud (µV)** | **Peak time/ Latencja (ms)** |
| study group/ grupa badana | controls/ grupa kontrolna | **p/ wartość p** | study group/ grupa badana | controls/ grupa kontrolna | **p/ wartość p** |
| Rod b-wave/ Fala b odpowiedzi pręcikowej | 80.99 ± 32.74 | 98.60 ± 27.84 | .03 | 110.15 ± 6.82 | 105.71 ± 8.03 | .01 |
| Rod-cone a-wave/ Fala a odpowiedzi pręcikowo-czopkowej | 143.29 ± 45.28 | 177.15 ± 48.38 | .002 | 24.35 ± 1.35 | 23.90 ± 1.07 | .14 |
| Rod-cone b-wave/ Fala b odpowiedzi pręcikowo-czopkowej | 363.67 ± 72.51 | 397.09 ± 67.48 | .03 | 48.25 ± 6.02 | 47.68 ± 2.83 | .02 |
| OPs wave index/ Indeks fal OP | 94.07 ± 29.95 | 114.46 ± 34.86 | .009 | - | - | - |
| Cone a-wave single flash/ Fala a odpowiedzi czopkowej | 28.04 ± 10.56 | 29.44 ± 8.03 | .96 | 16.79 ± 0.96 | 15.95 ± 1.20 | .0009 |
| Cone b-wave single flash/ Fala b odpowiedzi czopkowej | 81.76 ± 23.50 | 89.18 ± 22.88 | .19 | 32.85 ± 1.75 | 32.03 ± 1.64 | .02 |
| Cone b-wave 30-Hz flicker/ Fala b 30-Hz flicker | 64.44 ± 23.21 | 76.64 ± 18.63 | .01 | 32.72 ± 0.71 | 32.89 ± 1.06 | .08 |

*Statistically significant values were given in bold/ Wartości istotne statystycznie wytłuszczono.

Decrease in the amplitudes was identified in the ICAS group (p = .009). Accordingly, in the ICAS eyes, we recognized a significant decrease in the cone b-wave 30-Hz flicker amplitudes (p = .01) and a delay in the a-wave and b-wave single flash peak times (p = .0009 and p = .02, respectively).

Furthermore, to characterize the other risk factors that may influence the electrophysiological function of the retina and optic nerve, we investigated the association between full-field ERG amplitudes and peak times and several systemic risk factors and conditions. We determined that increasing extent of internal carotid stenosis was associated with longer rod b-wave (rs = .50, p = .009), rod-cone a-wave (rs = .45, p = .02), rod-cone b-wave (rs = .68, p = .0001), PERG P50 (rs = .60, p = .001) and N95 (rs = .50, p = .008) implicit times. Interestingly, the rod b-wave amplitudes and implicit times were dependent on the amount of cigarettes smoked. An increase in the smoking pack-years was associated with lower b-wave amplitudes (rs = -.41, p = .04 and rs = -.36, p = .02) in patients and controls, respectively) and longer rod b-wave implicit times (rs = .44, p = .02 in ICAS group). Similarly, the magnitudes of both the cone b-wave 30-Hz flicker and cone b-wave single flash amplitudes correlated with the amount of cigarettes smoked. An increase in the smoking pack-years was associated with lower cone b-wave single flash amplitudes (rs = -.35, p = .07 and rs = -.32, p = .007 in patients and controls, respectively) and cone b-wave 30-Hz flicker amplitudes (rs = -.39, p = .04 in ICAS group). Accordingly, in ICAS group, an increase in smoking pack-years was associated with longer rod-cone a-wave (rs = .41, p = .03) and b-wave (rs = .37, p = .06) implicit times.

**Discussion**

Ocular circulation is predominately controlled by autoregulatory mechanisms, which maintain retinal stability and, to a large extent, choroidal and optic nerve blood supply. In the case of carotid atherosclerosis and hemodynamically significant internal carotid artery stenosis, chronic cerebral hypotension may induce a downward shift in the cerebral and ocular vascular autoregulation. A major part of oxygen and glucose consumed by the retina is delivered by the choroid (9). The choroid has one of the highest blood flows in the body; however, it has no effective autoregulation. Consequently, the outer retina is more susceptible to hypoxia as a result of systemic ischemia (10). It has been demonstrated that patients with ICAS have thinner choroids compared with age-matched healthy individuals (11). Similarly, choroidal hypoperfusion as a result of ICAS resulted in multiple occlusions of the choriocapillaris and attenuated choroidal vessels (12). In contrast, retinal circulation through the central retinal artery is highly dependent on the blood concentration of O₂ and CO₂ and the pH levels in the inner retina (13, 14). Retinal flux may be increased in hypoxia by up to 336% (13), which is not possible in the choroid.

It has been demonstrated that full-field a- and b-wave ERG amplitudes are reduced in ocular ischemic syndrome compared with healthy eyes (4). More recently, Kofood at al. identified significant delays in all first-order summed implicit times and a clear trend towards reduced amplitudes in mfERG compared with healthy controls (15). In the present study, we demonstrated that even in the absence of the clinical signs of ocular ischemic syndrome, significant ICAS may be associated with abnormal retinal function. We identified significantly decreased amplitudes and prolonged peak times of scotopic b-waves and scotopic/photopic a-waves, as well as a reduced OP wave index in ICAS eyes compared with controls. It is widely accepted that the a-wave is primarily generated from photoreceptors and the b-wave is generated from bipolar cells (16). Oscillatory potentials (OPs) are small rhythmic waves superimposed on the ascending b-wave of the ERG, and amacrine and bipolar cells are directly or indirectly involved in their generation (17). Thus, our findings suggest that significant carotid stenosis mainly affects the rod-mediated response. It should be noted that the outer retina is particularly susceptible to hypoxic injury in the dark compared with the light, because oxygen consumption in photopic conditions represents only 60% of consumption.
in a scotopic environment (18, 19). Increased oxygen consumption is related to an increase in retinal blood flow from 40% to 70% following the transition from light to dark (20). Our full-field ERG results are in agreement with the findings of other groups (5).

Our findings indicated that ICAS may impair macular function, which was demonstrated by the significant reduction in the PERG P50 and N95 wave amplitudes. PERG has been used for assessing macular function in a clinical setting, because it is a focal response that reflects the activity of directly stimulated retinal areas and is thought to be correlated mainly with ganglion cell activity (21). The objective macular function assessment using PERG enables practical differentiation between maculopathy and optic neuropathy in different clinical settings (22). Most importantly, we found that increasing extent of internal carotid stenosis was associated with longer P50 and N95 implicit times. Our results are in keeping with previously published study performed with the use of mfERG in patients with unilateral carotid artery stenosis and without clinical eye disease. The study demonstrated significantly reduced N1P1 amplitude and delayed P1 latency in electroretinogram, which were correlated with an increasing grade of carotid artery stenosis (6). Accordingly, we found an increase in P100 wave latency which indicated that ICAS might be associated with impaired ganglion cell function of central retinal area. These findings may suggest that the macula is affected well before the onset of clinical eye disease. Our results indicate the importance of PERG testing in the evaluation and management of patients with asymptomatic severe ICAS to identify macular dysfunction secondary to ICAS-related ischemic conditions. Interestingly, an increased in P100 wave latency may indicate that ICAS may be associated with impaired ganglion cell function.

Remarkably, the magnitudes of both the ERG and PERG amplitudes were dependent on the amount of cigarettes smoked in our study. The effects of cigarette smoking on PERG amplitudes and implicit times in habitual smokers have been previously reported (23). There are several mechanisms by which cigarette smoking negatively affects the retina: increased oxidative stress, lipid peroxidation, and platelet aggregation, as well as a reduction in plasma high-density lipoprotein or antioxidant levels (24, 25).

In conclusion, our study demonstrated that retinal bioelectrical function is negatively affected by hemodynamically significant ICAS despite the absence of the objective clinical signs and symptoms of ocular ischemia. ERG and PERG testing appear to be valuable tools for detecting retinal ischemia secondary to severe carotid stenosis and may represent a clinically useful adjunct to standard algorithms in the management of asymptomatic ICAS patients, particularly as a part of eligibility assessment for carotid surgery.

References:


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