Relationship between Abnormalities in Neurophysiological Processes in Central and Peripheral Regions of the Retina and Clinical Parameters in Patients with Different Stages of Primary Open-Angle Glaucoma

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Abstract.

Primary glaucoma is a disease causing the greatest number of vision-related problems. Accelerated death of retinal ganglion cells and their axons which comprise the optic nerve as well as their involvement in the pathological process of neuroglia which provides nutrition for the optic nerve are considered as the main components of the glaucomatous process. The latest clinical research methods such as scanning laser polarimetry and optical coherence tomography measure structural changes at different functional levels of the retina and the optic nerve. According to literature data, structural changes in progressive glaucomatous optic neuropathy occur prior to apparent functional and clinical manifestations of the disease. In optical coherence tomography of glaucomatous eyes a scan of the macula is recommended to be performed using the Fast Macular Thickness Map protocol; it allows detecting the additional information on the pathological process. However, in a significant number of patients primary open-angle glaucoma can be very difficult to diagnose, and the nature and localization of the damage to eye structure as well as visual functions in patients with glaucoma need to be clarified. Neurophysiological method – electroretinography – allows us to study the processes at different morphofunctional levels of the retina as well as to clarify the localization and nature of pathological changes in the above-mentioned structures and to control dynamics of changes in the structures of primary damage at various stages of the glaucomatous process.

The objective of the research was to study the relationship between abnormalities in neurophysiological processes in central and peripheral regions of the retina and clinical parameters in patients with different stages of primary open-angle glaucoma.

Materials and methods. Complex clinical and neurophysiological study of eyes of 186 patients (358 eyes) with primary open-angle glaucoma was performed at the Filatov Institute of Eye Diseases and Tissue Therapy of National Academy of Medical Sciences of Ukraine. The main group included 81 (51.92%) females and 75 (48.08%) males with different stages of the glaucomatous process. The average age of patients was 56.8±4.26 years. Neurophysiological method – electroretinography using a RETI-3 scan multifocal ERG system (Roland Consult, Wiesbaden, Germany) - was used to diagnose the pathological condition.

Results. The strongest correlation was observed between neurophysiological processes occurring in neurons of the macular area – cone of the outer photoreceptor cell layer of the retina and parameters of retinal light sensitivity as well as the visual field index. According to the obtained results in patients with pre-glaucoma there was a direct correlation between the bioelectrical activity of the macular cone and mean deviation of the differential light sensitivity of the retina (r=0.56, p=0.05); in patients with mild primary open-angle glaucoma there was an inverse correlation between the duration of latent periods of neurons in the macular area and the visual field index (r=−0.35, p=0.02); in patients with advanced primary open-angle glaucoma there was a direct correlation between the duration of latent period of macular cone and intraocular pressure (r=0.41, p=0.034). In patients with advanced primary open-angle glaucoma there was an inverse correlation between the duration of latent period of neurons of the retinal photoreceptor cell layer in the macular area and the visual field index (r=−0.42, p=0.029) as well as an inverse correlation between the parameters of latent period of the second-order neurons in the macular area and those of the intraocular pressure (r=−0.69, p=0.00000024); a direct correlation between the parameters of latent period of the second-order neurons in the macular area and those of the intraocular pressure was also observed (r=0.47, p=0.009).

Keywords: electroretinography; retina; visual field; intraocular pressure; primary open-angle glaucoma

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**Problem statement and analysis of the recent research**

Glaucoma remains one of the more severe chronic progressive diseases being one of the leading causes of blindness in Ukraine and worldwide. The number of people with glaucoma worldwide reaches 70 million and in Ukraine more than 200 thousand people are diagnosed with this disease. The prevalence of glaucoma increases from 0.5% to 10% among different age groups and patients over 40 years of age have an increased risk of developing this condition [1]. Treatment for glaucoma (medications, laser, surgery) aims to save the patient’s vision for the remaining of his or her life. To achieve this goal, it is necessary to eradicate glaucoma or to prevent the progression of the glaucomatous process [2, 3].

Recently, much attention has been paid to the study of the retina in patients with primary open-angle glaucoma (POAG). Accelerated death of retinal ganglion cells and their axons which comprise the optic nerve as well as their involvement in the pathological process of neuroglia which provides nutrition for the optic nerve are considered as the main components of the glaucomatous process [4, 5, 6]. The latest clinical research methods such as scanning laser polarimetry and optical coherence tomography (OCT) measure structural changes at different functional levels of the retina and the optic nerve [7]. According to literature data, structural changes in progressive glaucomatous optic neuropathy (GON) occur prior to apparent functional and clinical manifestations of the disease. In OCT of glaucomatous eyes a scan of the macula is recommended to be performed using the Fast Macular Thickness Map protocol; it allows detecting the additional information on the pathological process. However, in a significant number of patients POAG can be very difficult to diagnose, and the nature and localization of the damage to eye structure as well as visual functions in patients with glaucoma need to be clarified.

Neurophysiological method – electroretinography – allows us to study the processes at different morphofunctional levels of the retina as well as to clarify the localization and nature of pathological changes in the above-mentioned structures and to control dynamics of changes in the structures of primary damage at various stages of the glaucomatous process [8, 9].

Neurophysiological methods – electroretinography – are not specific methods for studying the glaucomatous process; however they allow us to study the processes at different morphofunctional levels of the retina as well as to detect any pathological changes in the preclinical stage of the disease being very important for studying the pathogenesis and early diagnostics [10].

The objective of the research was to study the relationship between abnormalities in neurophysiological processes in central and peripheral regions of the retina and clinical parameters in patients with different stages of POAG.

**Materials and methods**

Complex clinical and neurophysiological study of eyes of 186 patients (358 eyes) with POAG was performed at the Filatov Institute of Eye Diseases and Tissue Therapy of National Academy of Medical Sciences of Ukraine. The main group included 81 (51.92%) females and 75 (48.08%) males with different stages of the glaucomatous process. The average age of patients was 56.8 ± 4.26 years.

The control group included 30 patients with mild-degree and moderate-degree emmetropia without POAG and was compared with the main group by gender, age, and other somatic diseases.

All patients underwent visometry, tonometry, tonography, refractometry, biomicroscopy, direct and indirect ophthalmoscopy, determination of mean deviation (MD) of the differential light sensitivity of the retina and the visual field index (VFI) by the data of computer perimetry using the Humphrey 750 Field Analyzer (HFA; Carl-Zeiss Meditec, Dublin, CA, USA), and optical coherence tomography with the use of SOCT Copernicus (Optopol, Zawiercie, Poland).

Neurophysiological method – electroretinography (ERG) using a RETI-scan multifocal ERG system (Roland Consult, Wiesbaden, Germany) – was used to diagnose the pathological condition.
Depending on research conditions ERG can be recorded from the entire retinal surface (the global or full-field ERG) and from multiple regions of the retina. The multifocal ERG which is the total bioelectrical potential of the peripheral region (Rod-response, dark phase) is used to assess the functions of rod system of the peripheral retinal region.

The macular retinogram is the bioelectrical potential which is recorded when stimulating macular area of the retina. The recording of macular retinogram differs from that of global ERG not only in the surface of the stimulated area but in qualitative composition of cellular element – ERG generators. The macular ERG is used to assess the function of the macular cone system.

The rhythmic ERG is the graphical representation of the bioelectrical processes occurring in the retina when stimulating with light stimuli of different frequencies. The difference in the lability of various types of photoreceptors became a physiological basis for determining functions of both photopic and scotopic system of the retina using the rhythmic ERG. Critical frequency of blinking generated by both rods and cones of the human retina is within the range of 10-25 and 50-100 Hz, respectively. Therefore, there are two amplitudes of the rhythmic ERG: low-frequency ERG (stimulation frequency is 4-14 Hz) and high-frequency ERG (stimulation frequency is 30 Hz and higher). Under appropriate conditions of recording (dark or light adaptation, stimulus intensity and colour) it is possible to obtain only cone or rode response. In addition, Müller glia are known to be unable to perceive a light rhythm with a frequency higher than 2-4 Hz; correspondingly, the rhythmic ERG is purely neutral response of the retina without the involvement of Müller glia in the electrogenesis. Only cones may perceive a light rhythm with a frequency of 30 Hz and higher without the involvement of buffer properties of Müller glia. This type of ERG has a photoreceptor nature with minimum contribution from the proximal retinal activity.

Each characteristic according to the data of electroretinography is represented by two parameters: the amplitude (measured in microvolts) and latent period (LP) (measured in milliseconds). The above-mentioned parameters are important for evaluating the dynamics of ERG as well as the degree of progression of the pathological process.

ERG results were compared with the results obtained in the control group, literature data [11] and the standards of the equipment and the laboratory where the research was conducted as in clinical practice there are no recognized data standards.

The main group included 156 (298 eyes) patients being divided into 4 subgroups:
- subgroup I included 42 (84 eyes) patients with pre-glaucoma;
- subgroup II included 48 (96 eyes) patients with mild glaucoma;
- subgroup III comprised 36 (65 eyes) patients with advanced glaucoma;
- subgroup IV included 30 (53 eyes) patients with severe glaucoma.

Subgroup I included patients with suspected glaucoma. The results of reoophthalmography, OCT (interocular difference in the width of the optic disk excavation by more than 20%), tonometry, (interocular asymmetry; variations in data of daily tonometry by 3-4 mm) in this group of patients differed from normal values by one or several indicators. Some of them had clinically confirmed POAG in their second eye as well as a family history of the condition.

Patients with end-stage POAG and high-degree emmetropia were excluded from the study.

Optic nerve disorders, macular dystrophy, hypertensive retinopathy, clouding of the optic media, diabetes mellitus, brain injury, acute and chronic disorders of cerebral circulation were not observed among patients of the main and control groups.

The obtained data were processed using Statistica 10 software, the average values of standard deviation, reliability of the differences – p>0.05 (paired test with bilateral distribution), the Spearman’s correlation coefficient.
Results and discussion

156 (298 eyes) patients of the main group and 30 (60 eyes) patients of the control one underwent clinical examinations as well as neurophysiological ones (the multifocal ERG, the macular ERG and the rhythmic ERG). Each eye was tested separately.

There was no statistically significant difference in gender, age and other somatic diseases between patients of both groups.

Clinical characteristics of patients of both groups were criteria for the diagnosis and clinical course of the disease. According to the obtained results between patients with advanced POAG and those with severe POAG there was a statistically significant difference in the width of the optic disk excavation, intraocular pressure and perimetric parameters (MD and VFI). Statistically significant difference in the parameters of MD and VFI was observed only between patients with mild POAG and those with advanced POAG as well as between patients with mild POAG and those of the control group. There was no statistically significant difference in any parameters between patients with pre-glaucoma and those with mild POAG as well as between patients with pre-glaucoma and those of the control group.

According to the results of the multifocal ERG (Rod-response, dark phase) in patients with pre-glaucoma the duration of LP of the b-wave was not statistically different from that in the control group; the amplitude of the a-wave reduced by 72.83%, p<0.05 compared to the control group. In patients with mild POAG the duration of LP of the b-wave was not statistically different from that in the control group; the amplitude of the a-wave reduced by 79.35%, p<0.05 compared to the control group. In patients with advanced POAG the duration of LP of the b-wave was also not statistically different from that in the control group; the amplitude of the a-wave reduced by 78.88%, p<0.05 compared to the control group. There was no statistically significant elongation of LP of the b-wave in patients with severe POAG; the amplitude of the a-wave reduced by 89.52%, p<0.05 compared to the control group.

Using the Spearman’s correlation coefficient we made the analysis of data of the main clinical parameters and ERG characteristics as well as established correlative relationships between them.

Correlation analysis of the obtained data revealed a direct correlation between parameters of the bioelectrical activity of the second-order neurons in the peripheral retina and mean deviation of the differential light sensitivity of the retina (r=0.53, p=0.009) in patients with pre-glaucoma.

According to the results of the macular ERG (Rod-response, light phase) in patients with pre-glaucoma there was no statistically significant difference in the duration of the a- and b-waves LP and the amplitude of the a-wave compared to the control group; the amplitude of the b-wave reduced by 76.52%, p<0.05 compared to the control group. In patients with mild POAG there was a statistically significant difference in the duration of the a- and b-waves LP by 28.01%, p<0.05 and 17.96%, respectively, compared to the control group; the amplitudes of the a- and b-waves reduced by 54.93% and 64.09%, respectively, p<0.05 compared to the control group. In patients with advanced POAG there was an elongation of the a- and b-waves LP by 30.17% and 84.80%, respectively, p<0.05 compared to the control group; the amplitudes of the a- and b-waves reduced by 56.32% and 54.34%, respectively, p<0.05 compared to the control group. Statistically significant elongation of the a- and b-waves LP was observed in patients with severe POAG - by 28.21% and 48.05%, respectively, p<0.05; the amplitudes of the a- and b-waves reduced by 66.07% and 74.08%, respectively, p<0.05 compared to the control group.

In patients with pre-glaucoma there was a direct correlation between the bioelectrical activity of neurons of the retinal photoreceptor cell layer in the macular area and MD (r = 0.44, p<0.03) and the VFI (r=0.35, p<0.04) as well; there was also a direct correlation between the second-order neurons of the retinal macular area and MD (r=0.34, p<0.04).
In patients with mild POAG there was a direct correlation between the bioelectrical activity of the retinal photoreceptor cell layer in the macular area and MD ($r=0.24$, $p<0.04$).

In patients with advanced POAG there was an inverse correlation between the duration of LP of neurons of the retinal photoreceptor cell layer in the macular area and the VFI ($r=0.42$, $p<0.029$) as well as an inverse correlation between the parameters of the bioelectrical activity of the retinal photoreceptor cell layer in the macular area and those of the intraocular pressure ($r=-0.69$, $p<0.0000024$); a direct correlation between the parameters of LP of the second-order neurons in the macular area and those of the intraocular pressure was also observed ($r=0.47$, $p<0.009$).

In patients with severe POAG there was a direct correlation between the parameters of LP of the second-order neurons of the retina and those of the intraocular pressure as well as an inverse correlation with MD ($r=-0.86$, $p=0.025$; $r=-0.89$, $p=0.02$).

According to the results of the rhythmic ERG (30 Hz Flicker) in patients at all stages of POAG latency values of the P1 peak were not statistically different from those in the control group; latency values of the N1 peak reduced significantly in patients with severe POAG by 45.07%, $p<0.05$ compared to the control group. According to the results of the rhythmic ERG (30 Hz Flicker) the amplitude of the N1-P1 peaks reduced by 69.75% in patients with pre-glaucoma, $p<0.05$; by 70.33% in patients with mild POAG, $p<0.05$; by 72.71% in patients with advance POAG, $p<0.05$; by 79.56% in patients with severe POAG, $p<0.05$ compared to the control group.

According to the obtained results in patients with pre-glaucoma there was a direct correlation between the bioelectrical activity of the macular cone and MD ($r=0.56$, $p=0.05$); in patients with mild POAG there was an inverse correlation between the duration of LP of neurons in the macular area and the visual field index ($r=-0.33$, $p=0.02$); in patients with advanced POAG there was a direct correlation between the duration of LP of macular cone and intraocular pressure ($r=0.41$, $p=0.034$).

When analyzing studied parameters nonparametric methods were used: Spearman’s correlation coefficients were calculated and their statistical significance was determined; the correlation coefficient herewith took values from +1 to -1.

The obtained data indicate a relationship between the bioelectrical activity of neurons of the retinal photoreceptor cell layer as well as the second-order neurons of the macular area and main clinical parameters such as retinal light sensitivity, visual field index and intraocular pressure in patients with POAG. Thus, abnormalities in neurophysiological processes in the macular area of the retina play an important role in the pathogenesis and progression of primary open-angle glaucoma.

**Conclusions**

Correlation between characteristics of photoreceptor cells (rods and cones) of the outer retinal layer, the second-order neurons of the retina (bipolars possibly containing horizontal and amacrine cells) as well as Müller glia of peripheral and central regions of the retina and clinical parameters of the functional state of visual analyzer in patients with different stages of primary open-angle glaucoma was firstly demonstrated using neurophysiological method – electroretinography in clinical practice.

Strong correlation between characteristics of the macular ERG and main clinical parameters such as mean deviation of the differential light sensitivity of the retina and the visual field index in patients with pre-glaucoma and mild POAG indicates that changes in neurophysiological processes occurring in neurons of the macular area of the retina are involved in the formation of clinical diagnostic parameters mentioned above at early stages of primary glaucoma development.

The intensification of the pathological process results in increased correlations between neurophysiological abnormalities in retinal neurons and the intraocular pressure parameters in patients with advanced and severe POAG. The impact of pathological neuronal changes on the formation of the parameter mentioned above also increases indicating the pathogenic nature of
neurophysiological retinal changes mentioned above. In patients with advanced POAG there was an inverse correlation between the parameters of the bioelectrical activity of the retinal photoreceptor cell layer in the macular area and those of the intraocular pressure \((r = -0.69, p = 0.0000024)\) as well as a direct correlation between the parameters of LP of the second-order neurons in the macular area and those of the intraocular pressure \((r = 0.47, p < 0.009)\).

The strongest correlation was observed between neurophysiological processes occurring in neurons of the macular area – cone of the outer photoreceptor cell layer of the retina and parameters of retinal light sensitivity, the visual field index, intraocular pressure. According to the obtained results in patients with pre-glaucma there was a direct correlation between the bioelectrical activity of the macular cone and mean MD \((r = 0.56, p = 0.05)\); in patients with mild POAG there was an inverse correlation between the duration of LP of neurons in the macular area and the VFI \((r = -0.33, p = 0.02)\); in patients with advanced POAG there was a direct correlation between the duration of LP of macular cone and intraocular pressure \((r = 0.41, p = 0.034)\).

The above-mentioned data are important for early diagnosis of studied pathology and formation of risk groups to provide timely ophthalmic care as well as to develop the complex of differentiated preventive and curative measures.

**References**