Neuroimaging Findings in Glaucoma (Proton Magnetic Resonance Spectroscopy Study)

Gazizova Ilmira[1*], Bogdan Andrey[2], Khomenko Yulia[3], Gromova Elena[4], Zainullina Svetlana[5], Gadzhiev Abdulla[6]

Abstract

The aim of the research is to determine metabolic changes in brain tissue by magnetic resonance spectroscopy (MRS) for patients who had primary open angle glaucoma (POAG) and to reveal a correlation between morphometric and functional alterations in the optic nerve and retina with basic MRS data. **Methods:** The study involved 24 patients with a diagnosis of POAG aged 58 to 80 years (mean age 73.2 ± 9.5 years). All patients underwent verification of the diagnosis of POAG, quantitative static perimetry (indexes were evaluated: MD - mean deviation), optical coherence tomography of the retina (assessment of the area of the neuro-retinal rim (NRR) and the thickness of the nerve fibers' layer of the retina (RNFL)). The 2D-PRESS (Point RESolved Spectroscopy) volume-selection method was used for multivoxel MRS, TE (Time Echo) = 144ms TR (Time Repetition) = 2s, the size of each voxel was 10 * 10 * 15mm. The region of interest included thalami (4 voxels in each thalamus) and occipital cortex; Cho/Cr, NAA/Cr and NAA/Cho were evaluated. **Results:** According to analysis of variance, the ratio of NAA/Cr in the right thalamus was associated with the POAG stage of the right eye ($F = 5.006$; $p = 0.0173$) and decreased with increasing of the disease stage. At the same time, there were no significant differences in different stages of POAG of the left eye ($F = 1.337$; $p = 0.285$). The ratio of NAA/Cr in the left thalamus did not differ depending on the POAG stage of the right eye ($F = 1.337$; $p = 0.285$) but depended on the stage of POAG of the left eye ($F = 6.245$; $p = 0.0074$). Thus, the eye POAG stage affected the ratio of NAA/Cr in the ipsilateral thalamus, but not in the contralateral one. It was also revealed that the ratio of NAA/Cr in the right occipital cortex correlated positively with the area of the NRR and MD of the left eye ($r = 0.54$; $p = 0.0069$ and $r = 0.54$; $p = 0.0260$, respectively). **Conclusions:** The decline of NAA/Cr ratio in thalamus and occipital cortex is observed in patients with primary open-angle glaucoma, which confirms the impairment of functional state of these brain structures and may indicate the neurodegeneration in the central part of visual system in patients with POAG. The correlations of the stage of primary open-angle glaucoma and the Cho/Cr ratio and NAA/Cr ratio in thalamus were revealed, which could have diagnostic and prognostic value in POAG. The dependence of the ratio of NAA/Cr in the thalamus and occipital cortex and morphometric data of the optic disc and the functional state of the retina was determined, which confirms the involvement of the optic tract in the POAG progression and its possible development.

Key Words: Primary Open Angle Glaucoma (POAG), Multivoxel Magnetic Resonance Spectroscopy (MRS), N Acetyl Aspartate, Thalamus, Visual Cortex (VC), Neurodegeneration.

DOI Number: 10.14704/nq.2020.18.10.NQ20227 **NeuroQuantology 2020; 18(10):13-21**

Relevance

Glaucoma is the most usual reason for irreversible blindness, around ninety million people in the world suffer from this disease, and the number of patients only increases every year (Tham et al., 2014).

Corresponding author: Gazizova Ilmira
Address: [1*]Federal State Budgetary Scientific Institution Institute of Experimental Medicine, Akademika Pavlova St., Saint Petersburg, Russia; [2]N.P. Bechtereva Institute of Human Brain of the Russian Academy of Sciences, Akademika Pavlova St., Saint Petersburg, Russia; [3]N.P. Bechtereva Institute of Human Brain of the Russian Academy of Sciences, Akademika Pavlova St., Saint Petersburg, Russia; [4]N.P. Bechtereva Institute of Human Brain of the Russian Academy of Sciences, Akademika Pavlova St., Saint Petersburg, Russia; [5]State Budgetary Institution of Health of the Republic of Bashkortostan City Clinical Hospital No. 10, Koltsevaya St., Ufa, Russia; [6]State budgetary institution of public health of the Leningrad region Vsevolozhsk clinical inter-District Hospital, Koltushskoe Sh., Vsevolozhsk, Russia.
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 04 July 2020 **Accepted:** 17 September 2020

Currently, more and more authors attribute primary open-angle glaucoma (POAG) to a group of neurodegenerative diseases, which are characterized by structural damage to the optic nerve and slowly progressive death retinal ganglion cells (RCG) (Nucci et al., 2013; Avdeev et al., 2014 Gazizova et al., 2016; Gazizova et al., 2019;). According to the latest data, glaucomatous damage is not limited only to GCR. It extends to the entire visual pathway up to the visual cortex, and even zones of alterations in the brain, which are not associated with the optic tract, are determined (Nucci et al., 2013; Engin et al., 2014; Duncan et al., 2014). Monitoring intraocular pressure (IOP) remains an important task in the treatment of POAG, but normalization of IOP does not prevent progressive loss of visual fields (Nucci et al., 2013; Engin et al., 2014). Despite numerous explorations of POAG, in most patients with a long course of the disease, even with normalized IOP, there is a progressive deterioration in visual function. Modern non-invasive research methods, such as structural and functional magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), are used to study neurodegenerative alterations in POAG (Menzler et al., 2011; Frezzotti et al., 2014; Lestak et al., 2014; Li et al., 2014). These techniques are able to detect the presence of degenerative changes not only in the optic nerve, but also in the subcortical center of the visual system, which is confirmed in morphological studies (Nucci et al., 2013). A more complete understanding of retrobulbar glaucomatous damage may provide an opportunity to conduct the most effective diagnosis of POAG and help to discover new therapeutic strategies for protecting the optic nerve.

Non-invasive neuroimaging methods also include magnetic resonance spectroscopy (MRS), which allows to quantify, identify and detect biochemical compounds or metabolites in brain tissue. MRS is used in a wide range of neurological diseases to assess the state of nervous tissue (Khomenko et al., 2019).

The proton MRS method allows to determine content of different metabolites in the nervous tissue in vivo that can contribute to the disclosure of the neurodegeneration mechanisms and have diagnostic value. MRS allows to evaluate the concentration of choline (Cho), myoinositol (mI), N-acetyl-aspartate (NAA), creatine (Cr), glutamate (Glx), lipids, lactate and some other substances (Fig. 1). NAA is derived from aspartic acid and synthesized in neurons and transferred along axons. The NAA decrease is considered as a reliable biomarker of neuronal dysfunction and death, which has been proven by histological studies. Cr - nitrogen-containing carboxylic acid, which is involved in energy metabolism in neural and muscle cells. The Cr decrease can be a marker of gliosis. The Cho resonance peak includes signals from phosphocholine, glycerophosphorylcholine and, to a small extent, acetylcholine and free choline. The Cho peak reflects the metabolism of cell membranes. The Cho concentration increase indicates enhanced membrane metabolism, which is possible in demyelination, remyelination, inflammation and gliosis. mI is found mainly in glial cells, it is a precursor of membrane phospholipids, and its concentration is associated with the syntesis or degradation of myelin (Barker et al., 2009).

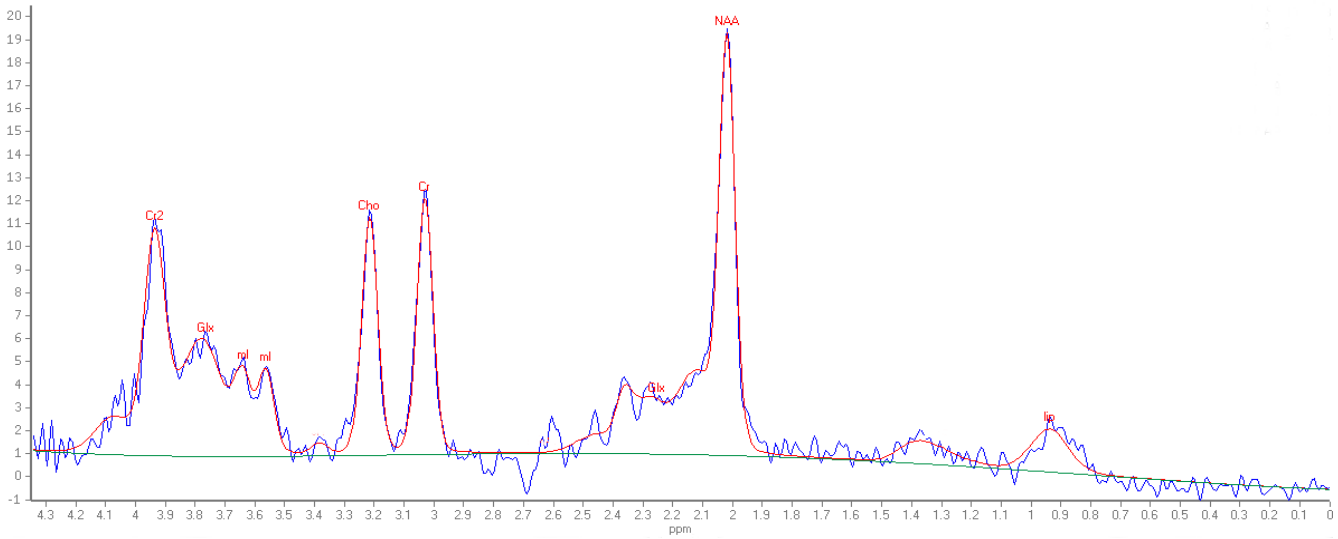

Fig. 1. MRS of neural tissue. NAA - N-acetyl aspartate, Cho - choline, Cr - creatine, mI - myoinositol, Glx - combined peak of glutamate and glutamine, lip - lipids. The x-axis represents the position of the peak in the spectrum in ppm (part per million), and the y-axis - the peak intensity

Despite the fact that the metabolite concentration is linearly proportional to the area under the peak on the MR spectrogram, the latter depends on the technical characteristics of the MR scanner, data processing algorithms, and protocol of acqusition (Li et al., 2003). Therefore, metabolites ratios are usually evaluated instead of absolute concentrations.

Usually, Cr is used as an internal referent, since its concentration in the neural tissue is considered to be relatively constant (Li et al., 2003), although there are a number of articles that argue (Ito-Shimizu et al., 2012). In addition, other metabolite ratios are used - NAA/Cho, mI/NAA, etc.

Thus, the aim of our research was to determine metabolits ratios in brain tissue using MRS in patients with POAG and to reveal correlations between morphometric and functional changes in the retina and optic nerve with brain metabolic changes.

Materials and Methods

The research involved 24 patients who had a diagnosis of POAG aged 58 to 80 years (mean age 73.2 ± 9.5 years). All patients underwent verification of the POAG diagnosis (biomicroscopy was performed using a slit lamp SL 115 (Carl Zeiss) and SN-2M (Takagi) with a 60 D lens for discoscopy, gonioscopy - a three-mirror Goldman lens, IOP was determined using a Maklakov tonometer (10.0 d)).

Functional changes in the field of view were evaluated by quantitative static perimetry using an HFA II field of view analyzer. Threshold programs were used to study the central region of the field 24-2 (indexes were evaluated: MD - mean deviation - reflects the average decrease in photosensitivity).

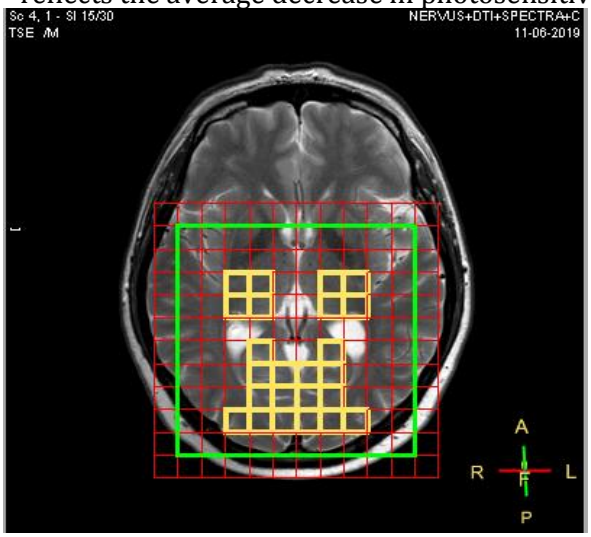

Fig. 2. The anatomical location of voxels when performing MRS

All patients underwent optical coherence tomography of the optic nerve and retina using an RTVue - 100 device (Optovue, USA) in order to calculate morphometric parameters. The maps of the retinal thickness in the MM5 mode and the parameters of the optic nerve disc (the area of the neuroretinal rim (NRR) and the thickness of the nerve fibers' layer of the peripapillary retina (LNFR)) were determined.

Multivoxel MRS

Multivoxel ^{1}H-MRS in supraventricular region was performed on Achieva 3T scanner (Philips), TE/TR=144/2000 ms (TE – time of echo, TR – repetition time), pulse sequence 2D PRESS (Point Resolved Spectroscopic Sequence). The PRESS is based on a double-spin echo experiment consisting of ninety degree radiofrequency (RF) pulse for excitation and two 180 degree refocusing pulses applied in frequency-selective way in the presence of a magnetic field gradient for the extraction of a planar slice. With the use of orthogonal slice orientations, the voxel volume for MRS is extracted as the intersection of the three slices. PRESS provides better signal to noise ratio in comparison with another spectroscopic sequences. The size of each voxel was 10 * 10 * 15mm.

The SpectroView software package (Philips) was used to evaluate the spectra. The region of interest included thalamus (4 voxels in each thalamus) and the occipital cortex (Fig. 2). NAA/Cho, NAA/Cr and Cho/Cr ratios in normal appeared brain tissue were analyzed. Data from different voxels was averaged within the right and left thalamus and the right and left occipital region. Data from voxels, containing both white and gray matter, were excluded from the analysis.

Statistical data processing was performed using the R language. The distribution normality was checked using the Shapiro-Wilk test. The analysis of variance was performed to study the differences in MRS data at different stages of glaucoma. Comparison of MRS data and visual function characteristics was carried out applying Spearman's rank correlation coefficient.

Results

In order to general analysis of MRS data in different stages of glaucoma all patients were separated into two subgroups (depending on the maximal stage of glaucoma on the left or right eye) 1 — with 1-2 stages and 2 — 3-4 stages. The subgroup with 1-2 glaucoma stage included 11 patients (average age 69.7 ± 10.7 years), the subgroup with 3-4 stage - 13 people (average age 76.3 ± 7.4 years), age difference between groups was statistically insignificant (p = 0.17).

Table 1 presents the data on the average metabolites, ratios in 2 subgroups of patients. It should be reported that the NAA content was decreased and the Cho/Cr ratio in the occipital cortex was increased in both subgroups (but it did not reach the level of statistical significance) compared with other areas of the cortex and white matter obtained in previous studies on other groups of patients (Khomenko et al., 2019). According to the published data, in the age over 60, the Cho/Cr ratio is 0.49 ± 0.12 (Ito-Shimizu et al., 2012). In patients examined in the present study it was slightly higher. These changes may indicate neurodegeneration in the cortex, although data on changes in the choline content in this, processes is contradictory: some researchers reported about its tendency to increase, others - the tendency to decrease (Khomenko et al., 2019).

In a model of experimental glaucoma in rats presented by Chan et al. (2009), change in the choline content was also detected. The design of this study did not include a control group; however, it is planned to do in future work to verify the assumption that changes of Cho and Naa in the occipital cortex in glaucoma are exist.

Table 1. Mean values of metabolite ratios according to MRS in patients with POAG

Group	NAA/Cr				Cho/Cr			
	Thalamus		Occipital cortex		Thalamus		Occipital cortex	
	RH	LH	RH	LH	RH	LH	RH	LH
1-2 st.	1,81±0,20	1,62±0,26*	1,72±0,17	1,64±0,14	1,12±0,15	0,96±0,14	0,59±0,07	0,63±0,09
3-4 st.	1,70±0,11	1,86±0,18	1,67±0,12	1,63±0,08	0,96±0,16*	1,02±0,19	0,59±0,06	0,68±0,09

Note: RH - the right hemisphere, LH - the left hemisphere;

*- the ratio was significantly decreased compared to the other group, p <0.05.

Comparison of MRS Data with the retina State Characteristics

To study the differences in MRS data at different stages of glaucoma more accurately, the analysis of variance was performed. Before the analysis of variance, the normality of the distribution was checked - the Shapiro-Wilk test, which showed that the distributions of the NAA/Cr ratio and Cho/Cr ratio in the thalamus and occipital cortex did not differ from the normal (p> 0.05).

Since there were few patients with stage 4 in the examined group of patients, stages 3 and 4 were combined into one subgroup. The following results were obtained.

Assessment of NAA Concentration in the Thalamus and Occipital Cortex at different Stages of Glaucoma

The ratio of NAA/Cr in the right thalamus depended on the stage of POAG of the right eye (F = 5.006; p = 0.0173), see Fig. 3. The NAA / Cr value decreased with an increase in the stage of the disease. At the same time, no significant differences were revealed with the stage of POAG of the left eye (F = 1.337; p = 0.285).

The ratio of NAA/Cr in the left thalamus did not differ depending on the stage of POAG of the right eye (F = 1.337; p = 0.285); however, it depended on the stage of POAG of the left eye (F = 6.245; p = 0.0074). The ratio of NAA/Cr was lower in the second stage of POAG compared with the first and third. Thus, the eye POAG stage affected mostly the NAA/Cr ratio in the ipsilateral thalamus; however, not in the contralateral one.

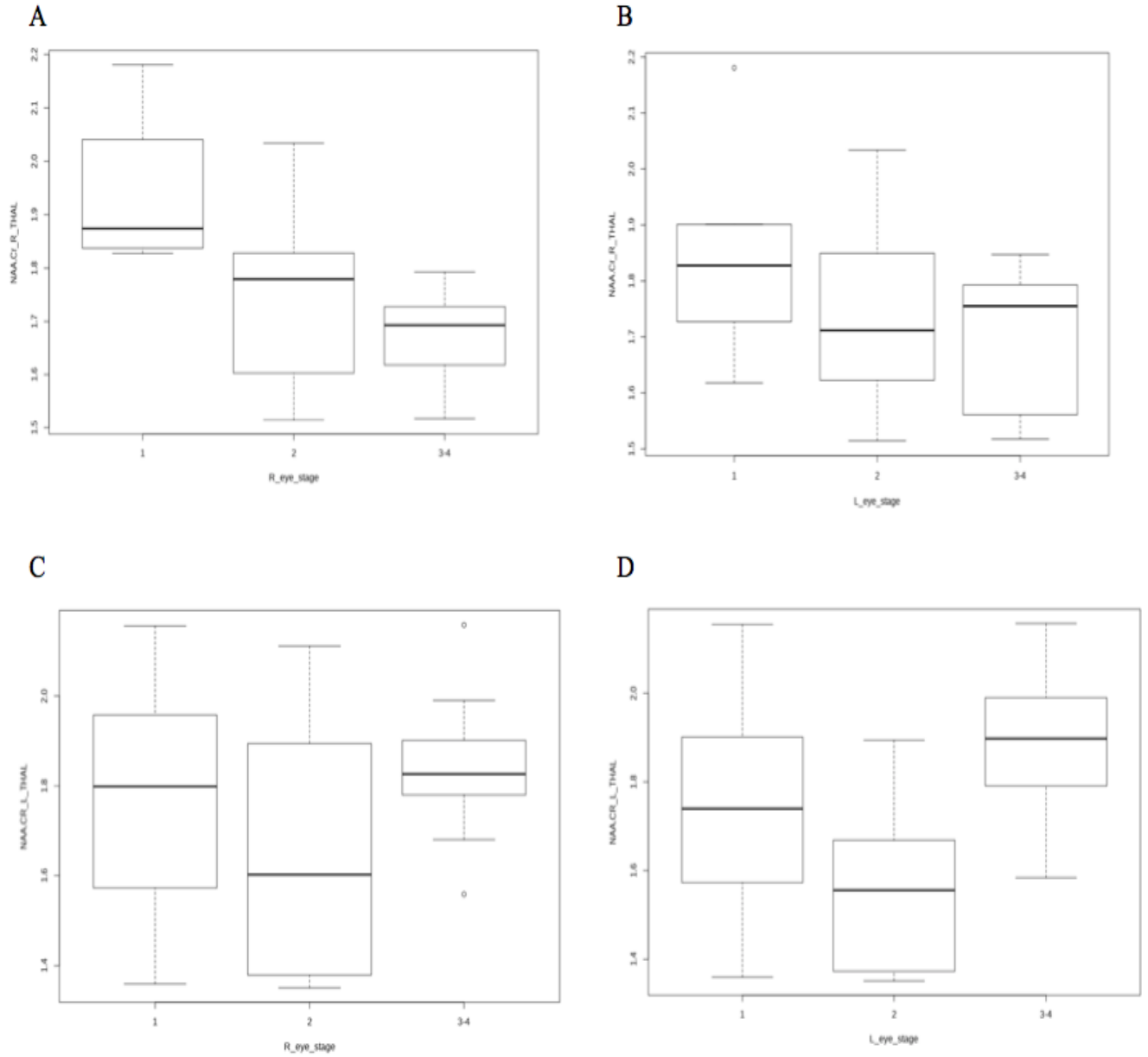

Fig. 3. The NAA/Cr ratio in the right and left thalami in subgroups with different POAG stages of the right (R) and left (L) eyes. NAA/Cr_R_THAL - NAA/Cr in the right thalamus, NAA/Cr_L_THAL - NAA/Cr in left thalamus. (A) Right eye POAG stage and NAA/Cr ratio in the right thalamus; (B) POAG stage of the left eye and the ratio of NAA/Cr in the right thalamus. (C) POAG Stage of the right eye and NAA/Cr ratio in the left thalamus; (D) POAG Stage of the left eye and NAA/Cr ratio in the left thalamus. The x-axis is POAG stage; the y-axis is metabolite ratio in the region of interest.

The fact that the ratio of NAA/Cr in the third POAG stage was higher than in the second is not entirely clear. Probably this could be a consequence of the Cr content increase. Although the concentration of creatine in brain tissue remains relatively constant, it still changes in a number of diseases, which is associated with the energy metabolism deterioration.

To clarify this fact, the comparison of NAA/Cho ratio in the same subgroups of patients was conducted, which did not differ in 3 subgroups ($F = 0.963$; $p = 0.399$). As can be seen at fig. 4, the NAA/Cho ratio almost did not differ in the subgroup with 2 and 3-4 stage of POAG of the left eye, while there are outliers in data - in 2 patients in with 3-4 stage this ratio was significantly higher than the group mean value.

Perhaps this is due to the individual characteristics, or some additional factor; however, due to the small sample size at the moment, it is impossible to draw a definite conclusion about it, and it is necessary to pay attention to this fact in further studies.

Comparison of NAA/Cr in the occipital cortex for patients with different stages of POAG of the right and left eye showed the absence of significant differences.

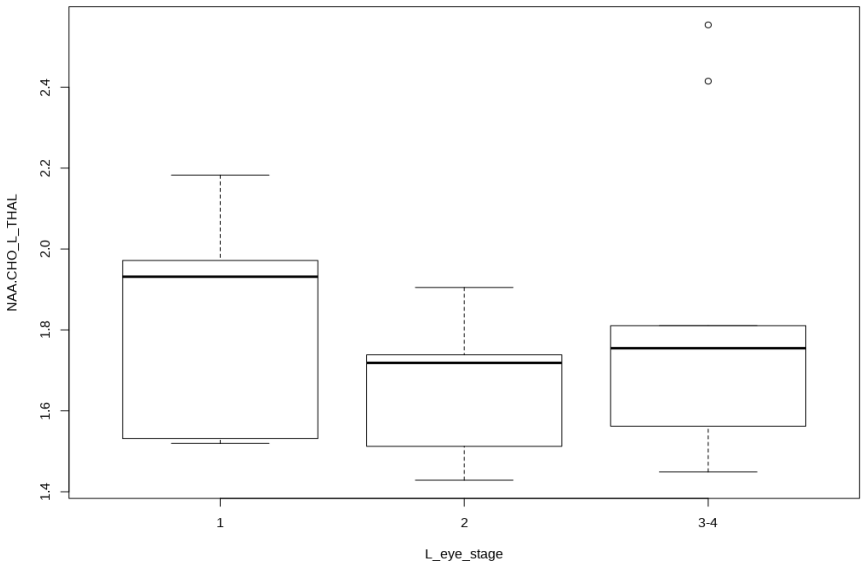

Fig. 4. The ratio of NAA / Cho in the left thalamus, depending on the varying stages of POAG of the left (L) eye. The abscissa axis is L_eye_stage; the ordinate axis is NAA.Cr_L_THAL

Assessment of Cho content in thalamus and occipital cortex at different stages of glaucoma

The Cho/Cr ratio in the right thalamus depended on the POAG stage of the right eye (F = 6.736; p = 0.0058) and the left eye (F = 3.8466; p = 0.0386) (see Fig. 4, the Cho/Cr ratio declined with the disease stage increasing.

The difference of Cho/Cr ratio in the left thalamus was not significant at different stages of POAG of the right (F = 0.146; p = 0.865) and left eye (F = 1.777; p = 0.195) (Fig. 5). Also, it could be seen that there are large dispersion of Cho/Cr ratio values in the left thalamus in the subgroup with 3-4 glaucoma stage, which is probably due to the individual characteristics of the patients, possibly associated with co-morbid pathology, which should be noted in further studies.

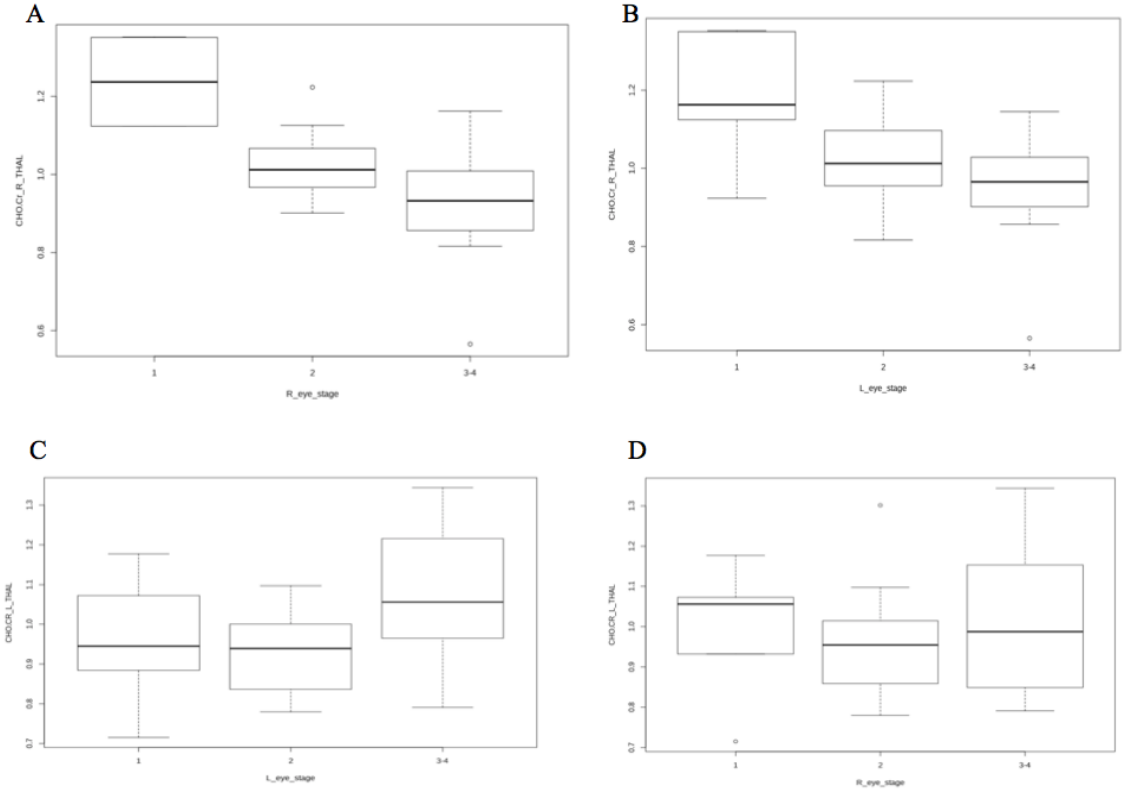

Fig. 5. The Cho/Cr ratio in the right and left thalami in subgroups with different stages of POAG of the right (R) and left (L) eyes. Cho/Cr_R_THAL - Cho/Cr in right thalamus, Cho/Cr_L_THAL - Cho/Cr in left thalamus. (A) POAG stage of the right eye and Cho/Cr ratio in the right thalamus. (B) POAG Stage of the left eye and Cho/Cr ratio in the right thalamus. (C) POAG stage of the left eye and Cho / Cr ratio in the left thalamus. (D) Stage POAG of the right eye and Cho / Cr ratio in the left thalamus. The x-axis is POAG stage; the y-axis is metabolite ratio in the region of interest.

Comparison of Cho/Cr in the occipital cortex in patients with different stages of POAG of the right and left eye showed the absence of statistically significant differences.

Correlation with metabolites ratios in the occipital cortex

The results of the correlation analysis of morphometric characteristics of the optic disc, the peripapillary retina and functional changes in the retina with the MRS data are presented in Table 2 and Fig. 6.

Table 2. Results of correlation analysis of NAA/Cr and Cho/Cr in the thalamus and occipital cortex with the retina state characteristic in patients with POAG of the right (R) and left (L) eyes

Condition of retina	Thalamus	Occipital cortex
The area of NRR:- R	NAA/Cr in LH (r=0,45; p=0,0355)	-
The area of NRR:- L	**Cho/Cr in LH (r=-0,56; p=0,0053)**	NAA/Cr in RH (r=0,54; p=0,0069)
Thickness of LNFR:- R	Cho/Cr in LH (r=0,48; p=0,0277)	-
Thickness of LNFR:- L	-	-
MD: - R	NAA/Cr in RH (r=0,56; p=0,0085) Cho/Cr in LH (r=0,49; p=0,0234)	-
MD: - L	-	NAA/Cr in RH (r=0,54; p=0,0260)

*LH - the left hemisphere, RH - the right hemisphere

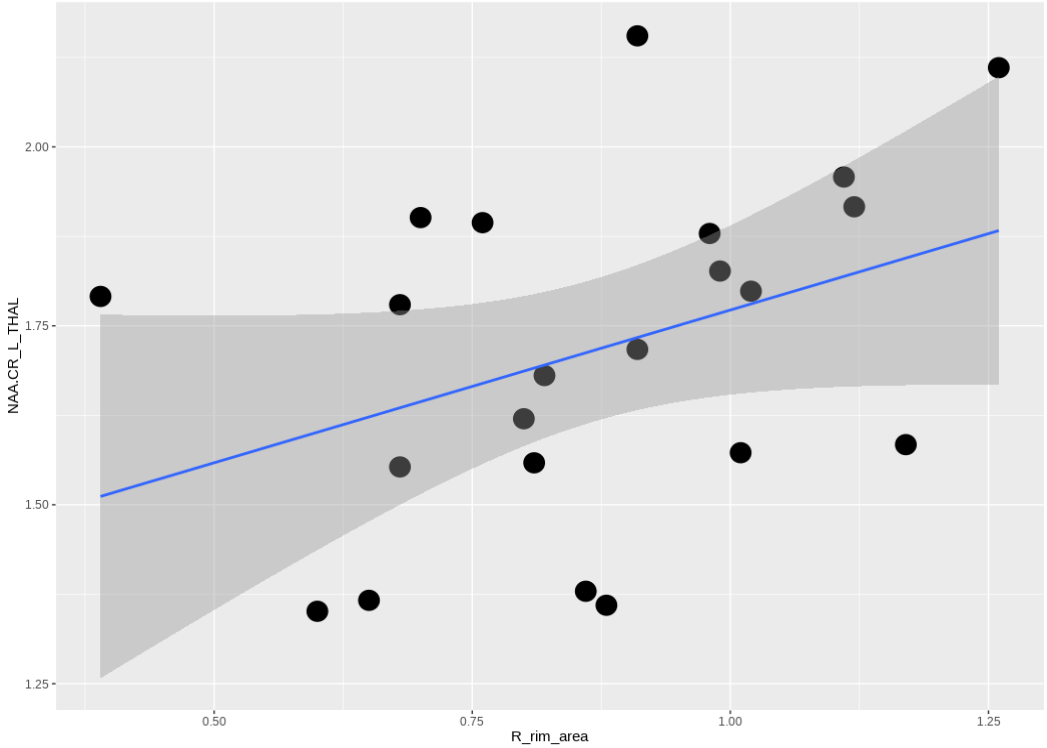

Fig. 6. A graphical representation of the correlation of the ratio NAA/Cr in the left (L) thalamus and the area of the neuroretinal rim of the optic nerve on the right (R). The x-axis is rim area; the y-axis is NAA/Cr

The obtained data indicate that with an increase of the POAG stage, the Cho/Cr ratio and NAA/Cr ratio decrease becomes more pronounced, confirming that POAG is accompanied by changes in the brain structures responsible for visual perception.

It was found that the NAA/Cr ratio in the right occipital cortex was positively correlated with the area of the NRR and MD of the left eye (r = 0.54; p = 0.0069 and r = 0.54; p = 0.0260, respectively). According to the literature, a decline in the NAA/Cr ratio occurs in patients with neurodegenerative diseases and dementia (Guo et al., 2018). This fact also could be a marker of neurodegeneration in the occipital cortex in POAG. Only NAA/Cr ratio in the left occipital cortex correlated considerably (r = -0.42; p = 0.0416) with the patients' age, (which is in the agreement with the fact that the NAA/Cr ratio in the cortex decreases with age) (Barker et al., 2009; Khomenko et al., 2019). No other correlations of morphofunctional retinal data with MRS data were found.

Discussion

In the present work, we studied the brain metabolism in patients with glaucoma using MRS and found that the content of Cho and NAA in the thalamus and occipital cortex changes in patients who had POAG. In addition, there are correlation dependences of the Cho/Cr and NAA/Cr ratios in thalamus and occipital cortex and morphometric data of the optic disc and the functional state of the retina.

As we know, only a few studies have been performed to study alterations in the concentration of metabolites in the visual pathway in patients with glaucoma using proton MRS (Boucard et al., 2007; Chan et al., 2009; Doganayet al., 2012; Zhang et al., 2013). Boucard et al. (2007) reported the earliest data for metabolic changes in 7 patients with glaucoma in comparison to twelve people without the disease. Researches did not detect considerable metabolic changes of Glx/Cr, NAA/Cr and Cho/Cr in the occipital lobe of patients in both groups applying the single-voxel ^{1}H-MRS method (Zhang et al., 2013).

In contrast, Doganay et al. (2012) found a statistically significant decrease in the Glx/Cr ratio in vitreous and lateral geniculate bodies, while studying single-voxel ^{1}H-MRS data of 29 patients with glaucoma and 13 healthy people; nevertheless, changes in NAA/Cr and Cho/Cr in lateral geniculate bodies were not detected. In addition, they found an increase of lactate in vitreous body in 11 patients with glaucoma.

In a research by Yan Zhang et al. (2013) alterations in the concentration of metabolites were detected in POAG applying multivoxel 1H-MRS in the geniculocalcaline tract and the striatum. 20 glaucoma patients participated in the research. Those were comparable in gender and age to twenty volunteers without glaucoma. A considerable decrease in Cho/Cr and NAA/Cr was detected in geniculocalcarin and striatum for patients with glaucoma in comparison to subjects without glaucoma (Zhang et al., 2013).

Using an experimental rat glaucoma model, Chan KC et al. (2009) used a rat glaucoma model to conduct a 1H-MRS research of the brain in animals and observed a considerable decrease in the Cho/Cr ratio in the left side of the visual cortex from induced glaucoma in comparison to the other side.

Guo et al. (2018) found that, compared to the control, the average Ins/Cr ratio (P = 0.001) was statistically lower, whereas the average Glx/Cr ratio (P = 0.003) in the primary visual cortex was considerably higher in patients with early POAG. Nevertheless, researchers did not observe evident difference in the average NAA/Cr ratio or the average Cho/Cr ratio between the group with POAG and the control group.

Also, in the research reported by Aksoy et al. (2019), it was shown that the NAA values obtained in lateral geniculate bodies in patients with confirmed and suspected glaucoma were lower than in healthy patients. Cho values in lateral geniculate bodies in patients with confirmed glaucoma were lower than in patients with suspected glaucoma and in the control group. A negative correlation was observed between the NAA values visual cortex and the ratio of excavation to the area of the optic disc in patients with glaucoma. In addition, a negative correlation was obtained between the age and thickness of the layer of retinal nerve fibers in patients with confirmed and suspected glaucoma.

MRS studies in glaucoma should be continued, including a group with suspected glaucoma and a group of patients with preperimetric stage of glaucoma to determine the possibility of using MRS in the early glaucoma diagnosis.

Conclusions

1. A decline of the NAA/Cr ratio in thalamus and occipital cortex was observed in patients with POAG, which confirms a decrease of the functional state and could be a marker of the neurodegenerative process in the central part of visual system in POAG.
2. Correlations of the stage of POAG and the NAA/Cr and Cho/Cr ratios in the thalamus were revealed, that could have diagnostic and prognostic significance in POAG.
3. The dependence of the NAA/Cr ratio in thalamus and occipital cortex and morphometric data of the optic disc and the functional state of the retina was determined, which confirms the involvement of the optic tract in the process of progression of POAG and its possible development.

References

Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng C. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology* 2014; 121(11): 2081-2090.

Nucci C, Martucci A, Cesareo M, Mancino R, Russo R, Bagetta J, Cerulli L, Garaci FG. Brain involvement in glaucoma: advanced neuroimaging for understanding and monitoring a new target for therapy. *Current Opinion in Pharmacology* 2013; 13(1): 128–133.

Avdeev R, Aleksandrov A, Bakunina N, Basinsky AS, Blyum EA, Brezhnev AY, Garkavenko VV. Model of manifestation and outcomes of primary open-angle glaucoma. *Clinical medicine* 2014; 92(12): 64-72.

Gazizova I, Avdeev R, Aleksandrov A, Basinskiy A, Blyum E, Brezhnev A, Galimova A, Gapon'ko O, Gar'kavenko V, Petrov S, Kuroedov A. Multicenter study of intraocular pressure level in patients with moderate and advanced primary open-angle glaucoma on treatment. *Investigative Ophthalmology and Visual Science* 2016; 57(12): 6470.

Gazizova I, Mazunin I, Malishevskaya T, Kiseleva O, Gadzhiev A, Rindzhibal Al. Mitochondrial DNA as a Factor of Glaucomous Optic Neuropathy's Development Mechanism. *Ophthalmology in Russia* 2019; 16(4): 479-486.

Engin KN, Yemisci B, Bayramoglu ST, Güner NT, Özyurt O, Karahan E, Öztürk C, Çagatay P. Structural and Functional Evaluation of Glaucomatous Neurodegeneration from Eye to Visual Cortex using 1.5T MR Imaging: A Pilot Study. *Journal of Clinical and Experimental Ophthalmology* 2014; 5(3): 341.

Duncan RO, Sample PA, Weinreb RN, Bowd C, Zangwill LM. Retinotopic organization of primary visual cortex in glaucoma: Comparing fMRI measurements of cortical function with visual field loss. *Progress in Retinal and Eye Research* 2007; 26(1): 38–56.

Menzler K, Belke M, Wehrmann E, Krakow K, Lengler U, Jansen A. Men and women are different: diffusion tensor imaging reveals sexual dimorphism in the microstructure of the thalamus, corpus callosum and cingulum. *Neuroimage* 2011; 54: 2557–2562.

Frezzotti P, Giorgio A, Motolese I, De Leucio A, Iester M, Motolese E, Federico A, De Stefano N. Structural and Functional Brain Changes beyond Visual System in Patients with Advanced Glaucoma. *Public Library of Science One* 2014; 9(8): e105931.

Lestak J, Tintera J, Svata Z, Ettler L, Rozsival P. Glaucoma and CNS. Comparison of fMRI results in high tension and normal tension glaucoma. *Biomedical Papers of the Medical Faculty of the University Palacky Olomouc, Czech Republic* 2014; 158(1): 144-153.

Li K, Lu C, Huang Y, Yuan L, Zeng D, Wu K. Alteration of Fractional Anisotropy and Mean Diffusivity in Glaucoma: Novel Results of a Meta-Analysis of Diffusion Tensor Imaging Studies. *Public Library of Science One* 2014; 9(5): e97445.

Khomenko YuG, Kataeva GV, Bogdan AA, Chernysheva EM, Susin DS. Cerebral Metabolism in Patients with Cognitive Disorders: a Combined Magnetic Resonance Spectroscopy and Positron Emission Tomography Study. *Neuroscience and Behavioral Physiology* 2019; 49(9): 1199–1207.

Barker PB, Bizzi A, De Stefano N, Gullapalli RP, Lin DM. *Clinical MR Spectroscopy: Techniques and Applications*. Cambridge University Press 2009.

Li BSY, Wang H, Gonena O. Metabolite ratios to assumed stable creatine level may confound the quantification of proton brain MR spectroscopy. *Magnetic Resonance Imaging* 2003; 21: 923–928.

Ito-Shimizu S, Maeno M, Yanashima K, Yamamoto S. Age and gender differences in metabolites of normal human occipital cortex measured by proton magnetic resonance spectroscopy（1H-MRS). *Chiba Medical Journal* 2012; 88E: 19-25.

Chan KC, So KF, Wu EX. Proton magnetic resonance spectroscopy revealed choline reduction in the visual cortex in an experimental model of chronic glaucoma. *Experimental Eye Research* 2009; 88(1): 65–70.

Guo L, Wang R, Tang Z, Sun X, Wu L, Wang J, Zhong Y, Xiao Z, Zhang Z. Metabolic Alterations within the Primary Visual Cortex in Early Open-angle Glaucoma Patients: A Proton Magnetic Resonance Spectroscopy Study. *Journal of Glaucoma* 2018; 27(12): 1046-1051.

Boucard CC, Hoogduin JM, Van Der Grond J, Cornelissen FW. Occipital proton magnetic resonance spectroscopy (1H-MRS) reveals normal metabolite concentrations in retinal visual field defects. *Public Library of Science One* 2007; 2(2): e222.

Doganay S, Cankaya C, Alkan A. Evaluation of corpus geniculatum laterale and vitreous fluid by magnetic resonance spectroscopy in patients with glaucoma; a preliminary study. *Eye (London)* 2012; 26(8): 1044–1051.

Zhang Y Chen X, Wen G, Wu G, Zhang X. Proton magnetic resonance spectroscopy ((1) H-MRS) reveals geniculocalcarine and striate area degeneration in primary glaucoma. *Public Library of Science ONE* 2013; 8(8): e73197.

Aksoy DÖ, Akkan JCU, Alkan A, Aralaşmak A, Temur HO, Yurtsever I. Magnetic Resonance Spectroscopy Features of the Visual Pathways in Patients with Glaucoma. *Clinical Neuroradiology* 2019; 29(4): 615-621.

*e*ISSN 1303-5150