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ASSESSMENT OF BIOMARKERS IN BIOLOGICAL FLUIDS AND NEUROIMAGING CHANGES IN PATIENTS WITH ALZHEIMER'S DISEASE AND GLAUCOMA

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Introduction. Alzheimer's disease (AD) and primary open-angle glaucoma (POAG) are gradually progressive neurodegenerative diseases leading to disability. According to literature data, POAG can be a predictor of AD development. Early diagnosis of these diseases contributes to a timely initiation of treatment and, as a result, a reduction in the disability of patients.

Objective. To study biomarkers of early diagnosis in biological fluids and neuroimaging changes based on the results of MR morphometry in patients with AD and POAG and to conduct their comparative analysis.

Materials and methods. In total, 90 patients with proven diagnosis of AD (group 1) and POAG (group 2) were examined. The study participants were divided into two groups according to their diagnosis: group 1 — 45 patients (9 (20%) men and 36 (80%) women) with AD; group 2 — 45 people (17 (37.8%) men and 28 (62.2%) women) with POAG. Neuropsychological testing included Mini-mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and a ten-words recall test. The beta-amyloid (Aβ) Aβ42/Aβ40 ratio in the blood and sirtuin Sirt1, 3, 5, and 6 in saliva were assessed by enzyme immunoassay (ELISA). In addition, MR morphometry of the brain was performed.

Results. In group 1, cognitive impairments (CI) reaching the degree of dementia were detected; in group 2, pre-dementia CI were observed ($p < 0.001$). According to the neuropsychological examination, similar changes were noted in both groups, in particular, memory impairment of the hippocampal type. The results of the blood and saliva ELISA with the determination of biomarkers in the groups under comparison did not reveal statistically significant differences. At the same time, the parameters of both volumes and thicknesses according to MR morphometry were lower in group 1 ($p < 0.05$), which may reflect neurodegenerative progression. In group 1, a direct correlation was found between a decrease in the saliva level of Sirt3 and a deterioration in direct reproduction (fifth reproduction) according to the ten-words recall test ($R = 0.43$; $p = 0.003$). Correlations between changes in neuropsychological parameters and MR morphometry data, including a decrease in the volume of the entorhinal cortex, were noted in both groups. In groups 1 and 2, a decrease in the Aβ42/Aβ40 ratio in blood plasma was associated with a decrease in the thickness or volume of the entorhinal cortex, which is common for both groups with different CI severity. Taking into account the association with neuropsychological and blood parameters, including in patients with pre-dementia CI from the POAG group, the determination of the volume and thickness of the entorhinal cortex can be regarded as a significant early marker of the neurodegenerative process.

Conclusions. The established association between the volume and thickness of the entorhinal cortex with neuropsychological and blood parameters, including in patients with pre-dementia CI from the POAG group, makes the determination of the volume and thickness of the entorhinal cortex a significant early marker of the neurodegenerative process. A comprehensive assessment of the results obtained by neuropsychological, laboratory, and neuroimaging diagnostic methods, as well as the search for diseases associated with the development of AD, such as POAG, are promising research areas, requiring larger cohort studies.

Keywords: Alzheimer's disease; dementia; cognitive impairment; primary open-angle glaucoma; MR morphometry; blood biomarkers; saliva biomarkers; sirtuins; Aβ42/Aβ40 ratio

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ОЦЕНКА БИОМАРКЕРОВ В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ И НЕЙРОВИЗУАЛИЗАЦИОННЫХ ИЗМЕНЕНИЙ У ПАЦИЕНТОВ С БОЛЕЗНЬЮ АЛЬЦГЕЙМЕРА И ГЛАУКОМОЙ

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Введение. Болезнь Альцгеймера (БА) и первичная открытоугольная глаукома (ПОУГ) относятся к постепенно прогрессирующим нейродегенеративным инвалидизирующим заболеваниям. В научной литературе отмечено, что ПОУГ может быть предиктором развития БА. Необходима ранняя диагностика данных заболеваний, которая будет способствовать своевременному назначению лечения и, как следствие, позволит снизить инвалидизацию пациентов.

Цель. Изучить биомаркеры ранней диагностики в биологических жидкостях и нейровизуализационные изменения по результатам МР-морфометрии у пациентов с БА и ПОУГ и провести их сравнительный анализ.

Материалы и методы. Обследовано 90 пациентов с установленным диагнозом БА и ПОУГ. Участники исследования были разделены на 2 группы в соответствии с диагнозом: группа 1 — 45 пациентов (из них 9 (20%) мужчин и 36 (80%) женщин) с БА; группа 2 — 45 человек (из них 17 (37,8%) мужчин и 28 (62,2%) женщин) с ПОУГ. Проведено нейропсихологическое тестирование: краткая шкала оценки психического статуса (MMSE), Монреальская шкала оценки когнитивных функций (MoCA), тест запоминания 10 слов. Всем пациентам определяли соотношение бета-амилоидов

(Аβ) крови Аβ42/Аβ40, в слюне — сиртуин Sirt-1,3,5,6 с проведением иммуноферментного анализа (ИФА), а также выполнялась МР-морфометрия головного мозга.

Результаты. В группе 1 были выявлены когнитивные нарушения (КН), достигающие степени деменции; в группе 2 — додементные КН ($p < 0,001$). По результатам проведенных нейропсихологических методик в двух группах были отмечены схожие изменения, в особенности нарушение памяти по гиппокампальному типу. Результаты проведенных ИФА крови и слюны с определением биомаркеров в двух сравниваемых группах не показали статистически значимых различий. При этом показатели как объемов, так и толщин по данным МР-морфометрии были ниже в группе 1 ($p < 0,05$), что может быть отражением прогрессирования нейродегенеративного процесса. В группе 1 выявлена прямая корреляционная связь снижения уровня Sirt3 в слюне с ухудшением непосредственного воспроизведения (5 воспроизведение) по тесту запоминания 10 слов ($R = 0,43$; $p = 0,003$). В обеих группах отмечены корреляционные связи между изменением нейропсихологических показателей и данными МР-морфометрии, в том числе уменьшением объема энторинальной коры. Как в группе 1, так и в группе 2 выявлено, что снижение соотношения Аβ42/Аβ40 в крови ассоциировалось с уменьшением толщины или объема энторинальной коры, что является общим для обеих групп с разной выраженностью КН. Учитывая наличие ассоциации с нейропсихологическими показателями и данными лабораторного анализа крови, в том числе и у пациентов с додементными КН из группы ПОУГ, определение объема и толщины энторинальной коры может быть расценено как значимый ранний маркер нейродегенеративного процесса.

Выводы. Выявлено наличие ассоциации с нейропсихологическими показателями и данными лабораторного анализа крови, в том числе и у пациентов с додементными КН из группы ПОУГ, в связи с чем определение объема и толщины энторинальной коры может быть расценено как значимый ранний маркер нейродегенеративного процесса. Комплексная оценка нейропсихологических, лабораторных и нейровизуализационных методов диагностики, а также поиск заболеваний, ассоциированных с развитием БА, таких как ПОУГ, является актуальным направлением, в связи с чем требуется дальнейшее проведение более крупных когортных исследований.

Ключевые слова: болезнь Альцгеймера; деменция; когнитивные нарушения; первичная открытоугольная глаукома; МР-морфометрия; биомаркеры крови; биомаркеры слюны; сиртуины; соотношение Аβ42/Аβ40

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INTRODUCTION

Alzheimer's disease (AD) is a gradually progressing neurodegenerative disease leading to disability and being the most common cause of dementia. At the same time, according to statistics, the number of patients with dementia is steadily increasing, which attracts the research attention toward the search for possible solutions [1].

Minimally invasive and noninvasive biomarkers of AD remain to be unavailable for widespread use, which frequently results in delayed diagnosis. When diagnosing AD in routine practice, the patient may already have clinical symptoms of dementia, which indicates the irreversible loss of 30–60% of the neurons in the temporal cortex, in particular the hippocampus [2]. This significantly limits the therapeutic capabilities of the clinician in affecting the course of the disease, leading to untimely initiation of treatment and irreversible changes. In addition, authors attribute the difficulties of creating a pathogenetically-based effective AD therapy to prescribing treatment only at the stage of clinical manifestations. At the same time, pathological proteins, in particular beta-amyloid (Aβ) that triggers the AD continuum, begin to accumulate long before the primary clinical symptoms of the disease appear [1]. This highlights the relevance of timely AD diagnosis and explains the current interest in identifying early diagnostic biomarkers and searching for other pathologies and conditions that may trigger or be associated with AD development.

Various studies have shown that AD is often associated with glaucoma, another progressive neurodegenerative disabling disease common among the population. About 74% of the patients suffer from primary open-angle glaucoma (POAG), which is characterized by thinning of the retinal nerve fiber layer and peripheral vision loss, up to complete blindness [3]. Glaucoma is assumed to be a disease that occurs in the brain, although being clinically manifested as an ophthalmological pathology. This is confirmed by the growing amount of information about retinal damage as a result of retrograde transynaptic degeneration caused by neurodegenerative processes [4]. Recent observational cohort studies have confirmed that glaucoma is a risk factor in the development of dementia among the adult population [5].

AD and POAG share numerous similarities, both in clinical and pathophysiological aspects. Thus, both diseases are associated with cognitive impairments (CI) of a neurodegenerative nature with damage to memory functioning as one of the most important cognitive domains [6].

A sufficient number of studies have confirmed the presence of similar links in the pathogenesis of AD and POAG. The cardinal signs in the pathogenesis of AD are extracellular accumulation of Aβ and intracellular deposits

of hyperphosphorylated tau protein (p-tau), which leads to progressive death of neurons. Aβ and p-tau accumulation in the main type of retinal cells — ganglion cells (GCs), affected by POAG, along with concomitant inflammation indicate coincident pathological processes in AD and POAG [3]. Therefore, Aβ and p-tau continue to be actively studied as early diagnostic biomarkers of these two diseases.

The gold standard of lifetime diagnosis of AD involves determination of markers in the cerebrospinal fluid (CSF) using positron emission tomography (PET) of the brain. However, this approach fails to meet the criteria of widespread availability and minimally invasiveness, making the search for other early diagnostic biomarkers for AD highly relevant. It should be emphasized that, since 2023, the revised AD criteria of the National Institute for Aging and the American Alzheimer's Association (National Institute on Aging, NIA-AA) have already included more widely available and less invasive accurate blood tests. Thus, the following are currently indicated as the main AD biomarkers in blood plasma: p-tau 217, the ratio of p-tau 217/np-tau 217 [7].

The Aβ42/Aβ40 blood plasma ratio is increasingly attracting attention as an important diagnostic indicator. Investigations showed that a lower Aβ42/Aβ40 ratio in blood plasma corresponded to a higher level of amyloid cortical load. Clinically and according to neuropsychological testing, a more pronounced cognitive dysfunction was observed, followed by an increased risk of dementia [8, 9]. Research into the diagnostic accuracy of this biomarker is underway, making the study of the Aβ42/Aβ40 ratio in the blood of POAG patients particularly relevant.

In addition to the classical amyloid theory of pathogenesis, which explains the development of AD and, to a lesser extent, POAG, other pathogenetic links should also be investigated. Recent studies into the processes underlying neurodegenerative diseases have shown that neuron death is influenced by a number of factors, including excitotoxicity, oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis. Various cell groups of the body, including the central nervous system, comprise a family of sirtuin proteins (Sirt) that are involved in almost all of the above links; disruption of the Sirt contributes to the development of degenerative processes.

Seven mammalian sirtuins — Sirt 1–7 — are involved in the regulation of metabolism in many tissues, presumably playing an important role in the pathology of AD and POAG [10]. For example, the induction of Sirt1 expression is believed to weaken neuron degeneration and death in AD animal models. Thus, the authors investigating ophthalmological pathologies [11] found that increased Sirt1 expression protects against diseases associated with eye damage due to oxidative stress, including optic nerve degeneration in patients with glaucoma. Sirt3 plays a protective role in AD and ophthalmological pathology, ensuring the normal functioning of mitochondria [12]. In addition, Sirt6 is known to alter neurogenesis in the hippocampus in adults, affecting the number of glial and neuronal cells, and thus may also contribute to the development of AD. A study of Sirt in the pathogenesis of glaucoma found that Sirt6 is highly expressed in GCs. Removal of Sirt6 in GCs led to a progressive loss of these cells and optic nerve degeneration [13]. In this context, the study of Sirt in biological fluids as early diagnostic biomarkers for AD and POAG can be considered as an important research area.

Neuroimaging, MR morphometry in particular, is another essential element in the early diagnosis of AD. Interestingly, similar such changes were observed in AD and glaucoma. Thus, according to a number of authors [14, 15], glaucoma affects not only the central visual cortex, but also other areas of the brain (e.g., the temporal lobes) that intersect with the areas affected in patients with AD, which also suggests a relationship between the two diseases.

This study is aimed at studying early diagnostic biomarkers in biological fluids and neuroimaging changes based on the results of MR morphometry in patients with AD and POAG followed by their comparative analysis.

MATERIALS AND METHODS

The research sample included 90 patients with confirmed diagnoses of AD or POAG, aged from 40 to 90 years, who are native speakers of the Russia language. The patients were lucid, without pronounced impairments of motor (on the Medical Research Council (MRC) Scale for Muscle Strength at least 4 points in the leading arm) and speech functions, without chronic diseases decompensation and other clinically significant neurological pathologies, history of mental disorders, without the brain MRI absolute contraindications and anxiety-depressive disorders according to the Hospital Anxiety and Depression Scale (HADS) [16, 17].

Following diagnostics, the study participants were divided into two groups: group 1 — 45 patients (9 men and 36 women, 20% and 80%, respectively) with AD; group 2 — 45 people (17 men and 28 women, 37.8% and 62.2%, respectively) with POAG. There were no statistically significant age differences when comparing the two groups: in group 1, the average age was 71 [66; 77] years, compared to 66 [61; 71] years in group 2.

The neurological status all patients was assessed. The state of cognitive functions was evaluated using a set of standard neuropsychological techniques. The Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used for integrative assessment of cognitive functions. The MMSE scale includes the following indicators: orientation, registration, attention and calculation, recall, and language. The maximum number of points on the MMSE scale is 30. The scale was used as a screening tool for cognitive impairments (CI): 28–30 points — normal, 25–27 points — moderate CI, 24 points and less points — severe CI (dementia). The MoCA scale is more sensitive for the diagnosis at the stage of moderate CI. On the MoCA scale, the following were assessed: executive and visuospatial function (include clock drawing test), naming, attention, language, abstraction, delayed recall, orientation. The maximum score on the MoCA scale was 30 points; the total score of less than 26 was regarded as CI [18, 19]. Auditory and verbal memory was assessed using a ten-words recall test (A.R. Luria). Normally, after the first memorization, the patient should reproduce

at least five words, after the 5th memorization — at least 9 words. The difference between the last immediate and delayed reproduction in healthy individuals is usually no more than one word [20]. In order to include patients with anxiety and depressive disorders in the study, the Hospital Anxiety and Depression Scale (HADS) was used during the initial screening.

Blood and saliva samples were collected from all the patients. For biomarker assessment of the Aβ42/Aβ40 ratio in blood plasma, enzyme immunoassay kits were used: for determining of beta-amyloid peptide 1–40 (CEA864Hu Enzyme-linked Immunosorbent Assay Kit For Amyloid Beta Peptide 1–40 (Ab1–40) Cloud-Clone Corp) and for determining of beta-amyloid peptide 1–42 (CEA946Hu Enzyme-linked Immunosorbent Assay Kit For Amyloid Beta Peptide 1–42 (Ab1–42)).

For saliva biomarker assay, enzyme immunoassay kits were used: Sirt1 (SEE912Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin1 (Sirt1)), Sirt3 (SEE913Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 3 (Sirt3)), Sirt5 (SEE915Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 5 (Sirt5)), Sirt6 (SEE916Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 6 (Sirt6)).

For laboratory tests, blood was taken from the peripheral vein using a vacuum system into Vacutest vacuum tubes with K3EDTA (4 mL) anticoagulant in the morning on an empty stomach. Tubes with a blood sample (no later than 30 min) were subjected to centrifugation. Blood for plasma production was centrifuged on an Awel CF108-R centrifuge for 15 min at 4000 rpm at a temperature of 2–8°C. After centrifugation, aliquoting was performed into 1 mL Eppendorf tubes, after which they were placed in a cryostat for subsequent freezing and storage at a temperature of minus 80°C for further laboratory analysis.

Saliva was collected on an empty stomach after brushing teeth in a plastic centrifuge tube (2–3 mL). To obtain the filler liquid, the biomaterial tube was centrifuged for 20 min at 4000 rpm. The filler liquid was taken using a Pasteur pipette and transferred to 1.5 mL Eppendorf tubes. Prior to the laboratory examination, the samples were stored in a cryostat in a frozen state at a temperature of minus 80°C.

All subjects underwent an MRI scanning of the brain followed by morphometric processing. The studies were performed on a 3.0 Tl Discovery MR750w tomograph (GE Healthcare, USA) using a 32-channel head coil. The scanning took place according to a single protocol for all participants. T1-, T2-weighted images, DWI, DWI, and T2-FLAIR were used to evaluate the brain structure and their selection for the study. T1-weighted images (when the patient was turned on) were used for morphometry. The IR-FSPGR sequence with an isotropic voxel of 1×1×1 mm was used. Parameters: TR 7.7 s, TE with a Min Full optimization, deviation angle 11°, bandwidth 31.25 Hz. The processing was performed automatically in the FreeSurfer software, which generated maps of gray and white matter based on the Desikan-Killiany and Destrieux atlases [21–24]. A volumetric assessment of brain regions and measurement of the thickness of various cortical regions were also performed.

The statistical analysis was performed using the RStudio environment, version 2023.09.1 Build 494, and the R programming language, version 4.3.2. The following statistical indicators were calculated for quantitative variables: arithmetic averages, standard deviations ($M \pm SD$), median and quartiles (Me [$Q1$; $Q3$]. When analyzing the differences between the two groups, the parametric Student's criterion or the nonparametric Mann–Whitney criterion were used for quantitative variables. Depending on the normality of data distribution, the Pearson correlation coefficient or Spearman correlation coefficient was used to study the relationships between the two quantitative variables. The qualitative variables between the groups were compared using the χ^2 (Chi-squared) criterion or the exact Fisher criterion if the expected frequencies were less than 5. All the differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS

The conducted assessment of cognitive functions revealed a statistically significant CI of varying severity in patients of both groups. Thus, according to the MMSE scale in group 1, the indicator was 18.6 ± 4.8 points, which corresponds to severe CI (dementia); in group 2 — 27.8 ± 2.1 points, which corresponds to moderate CI ($p < 0.001$). The overall score according to the MoCA scale in group 1 was also lower and amounted to 15.1 ± 4.4, which corresponds to severe cognitive impairment (dementia), while in group 2 moderate CI was also noted — 24.6 ± 1.9 points ($p < 0.001$). Both groups of patients were characterized by impairments in the cognitive domain of memory, with difficulties in reproducing (especially delayed) and recognizing the previously presented material, reflecting a defect in capturing, consolidating, and extracting information.

In the ten-words recall test, the following results were obtained: in group 1, with direct playback (1 and 5 playback), 2.6 ± 0.9 and 4.4 ± 1.4 words, respectively; in group 2, with direct playback (1 and 5 playback) — 5 ± 1.2 and 8 ± 1.6 words, respectively. In both groups, there was a decrease in direct reproduction of words, while in group 1, the decrease was more significant with both 1 and 5 reproductions ($p < 0.001$). With delayed playback of 10 words in the memorization test, a more pronounced decrease in the level of memorization was also recorded in group 1 compared to patients from group 2: 1.3 ± 1.5 words and 6.4 ± 2 words, respectively ($p < 0.001$).

Table 1 shows the results of blood and saliva ELISA with biomarker assessment in the two compared groups. According to the comparative analysis, no statistically significant differences of biomarkers in biological fluids were obtained. At the same time, it should be noted that the blood plasma Aβ42/Aβ40 ratio of patients from group 1 is lower than in patients from group 2.

Table 2 shows the results of MR morphometry in the two compared groups. Statistically significant differences ($p < 0.05$) were revealed for all the studied MR morphometry parameters. At the same time, both the volume and thickness of brain structures were lower in group 1.

In the course of the study, an assessment of correlational relationships was carried out. In group 1, a decrease in the level of Sirt3 in saliva correlated with a deterioration in direct reproduction (fifth reproduction) in the ten-words recall test ($R = 0.43$; $p = 0.003$). In group 2, there were no correlations between the studied neuropsychological parameters and biomarkers in biological fluids ($p > 0.05$).

Unlike biomarkers in biological fluids, the analysis of the relationship between cognitive indicators and the results of MR morphometry revealed the presence of a significantly larger number of correlations.

In group 1, a decrease in the overall MMSE score was associated with a decrease in the volume of the right ($R = 0.31$ $p = 0.038$) and left hippocampus; the right ($R = 0.41$; $p = 0.006$) and left entorhinal cortex ($R = 0.34$; $p = 0.022$); the thickness of the left cingulate gyrus ($R = 0.36$; $p = 0.017$) according to MR morphometry data. Two of these neuroimaging parameters were correlated with the MoCA school: the volume of the left hippocampus ($R = 0.31$; $p = 0.04$), and the thickness of the left cingulate gyrus ($R = 0.36$; $p = 0.016$).

In group 2, a decrease in the overall score on the MMSE scale correlated with the volume of the right entorhinal cortex ($R = 0.39$; $p = 0.007$); on the MoCA scale, the volume of the right entorhinal cortex ($R = 0.34$; $p = 0.024$), the volume of the left ($R = 0.44$; $p = 0.003$) and the right cingulate gyrus ($R = 0.37$; $p = 0.012$).

In patients with AD (group 1), the results of the ten-words recall test (decreased direct reproduction) were correlated with a decrease in the volume of the left entorhinal cortex ($R = 0.31$; $p = 0.04$); in group 2 — with a decrease in the volume of the right entorhinal cortex ($R = 0.48$; $p < 0.001$). A decrease in delayed playback according to the ten-words recall test in patients from group 1 with AD was associated with a decrease in the volume of the right hippocampus ($R = 0.34$; $p = 0.021$), the volume of the left hippocampus ($R = 0.5$; $p < 0.001$), the volume of the left entorhinal cortex ($R = 0.32$; $p = 0.035$), the thickness of the left the entorhinal cortex ($R = 0.43$; $p = 0.003$).

A correlation analysis of the parameters studied in biological fluids and the obtained MR morphometry data was performed for the two examined groups. In group 1, a correlation was found between a decrease in the blood Aβ42/Aβ40 ratio and a decrease in the following indicators according to the results of MR morphometry of the brain: the volume of the right hippocampus ($R = 0.33$; $p = 0.028$), the thickness of the right ($R = 0.37$; $p = 0.012$) and the left entorhinal cortex ($R = 0.38$; $p = 0.01$), thickness of the right

Table 1. Comparative characteristics of biomarkers in biological fluids in two groups

Parameter	Group 1, $n = 45$	Group 2, $n = 45$	p-value
Aβ42/Aβ40 ratio	0.129 ± 0.097	0.164 ± 0.106	0.104
Sirt1, ng/mL	0.22727 ± 0.1649	0.21932 ± 0.18647	0.648
Sirt3, ng/mL	0.064 ± 0.022	0.086 ± 0.127	0.601
Sirt5, ng/mL	0.0191 ± 0.0151	0.0192 ± 0.017	0.886
Sirt6, ng/mL	0.1342 ± 0.0694	0.1182 ± 0.0586	0.398

Table prepared by the authors using their own data

Note: Data is presented in the form of a mean value and a standard deviation ($M \pm \delta$).

Table 2. Comparative characteristics of MR morphometry results in two groups

Parameter	Group 1, $n = 45$	Group 2, $n = 45$	p
Volume, mm^3			
Right hippocampus	3208.2 ± 486.3	3862.4 ± 630.2	<0.001
Left hippocampus	3100.2 ± 523.8	3824.5 ± 610.6	<0.001
Right entorhinal cortex	1284.6 ± 545.4	1633.4 ± 379.1	<0.001
Left entorhinal cortex	1238.4 ± 477	1778 ± 389.7	<0.001
Right cingulate gyrus	1972 ± 331.3	2194.2 ± 359.8	<0.001
Left cingulate gyrus	2170.9 ± 283.5	2344.8 ± 361.5	0.002
Thickness, mm			
Right entorhinal cortex	2.7011 ± 0.5304	3.2073 ± 0.3903	<0.001
Left entorhinal cortex	2.4711 ± 0.5169	3.1506 ± 0.3561	<0.001
Right cingulate gyrus	2.0273 ± 0.188	2.2267 ± 0.2379	<0.001
Left cingulate gyrus	2170.9 ± 283.5	2344.8 ± 361.5	<0.001

Table prepared by the authors using their own data

Note: Data is presented in the form of a mean value and a standard deviation ($M \pm \delta$).

cingulate gyrus (R = 0.3; p = 0,042). In group 2, a correlation was found between a decrease in the blood Aβ42/Aβ40 ratio and a decrease in the volume of the right entorhinal cortex (R = 0.31; p = 0.037). There were no correlations between the levels of Sirt1, 3, 5, and 6 in saliva and the results of MR morphometry.

DISCUSSION

According to a number of publications, the relationship between the development of AD and POAG has been known for quite a long time. Thus, changes in the eye reflect pathological processes in the brain, including those associated with neurodegenerative diseases such as AD, have been identified. Due to the presence of several characteristics in common, AD and glaucoma are hypothesized to be manifestations of the same pathological process with heterogeneous manifestations. Indeed, the frequency and severity of both conditions increase with age, and the prevalence of glaucoma is higher in patients with AD than in the general population [5, 6]. The above highlights the relevance of studying the relationship between these two diseases, including with respect to the search for common biomarkers.

In this work, we set out to assess the cognitive sphere of patients in two study groups and confirmed the previous data that patients with both POAG and AD demonstrate CI of various levels. At the same time, CI were less pronounced in patients with POAG. However, despite significant statistical differences between groups 1 and 2 in the severity of cognitive dysfunction, similar impairments were noted according to the results of neuropsychological techniques. In particular, this concerns memory impairment of the hippocampal type, which demonstrates the neurodegenerative nature of CI for both diseases [6].

In our study, ELISA was used to determine the Aβ42/Aβ40 ratio in blood plasma; no statistical differences were found between the two groups. However, in group 1, AD patients had a lower plasma ratio of Aβ42/Aβ40. Our results agree well with other studies that consider POAG, a neurodegenerative ophthalmological disease, to be a possible predictor of AD development [25–27]. Our data are consistent with most of the studies conducted, which postulate that a lower Aβ42/Aβ40 ratio in blood plasma is associated with a higher amyloid cortical load according to PET data and an increased risk of dementia in AD [9]. At the same time, higher Aβ42/Aβ40 ratios in patients with POAG can be explained by the difference in the severity of cognitive deficits.

The diagnostic accuracy of AD biomarkers in the blood is still inferior to those in cerebrospinal fluid, having a number of disadvantages and thus requiring further work in this direction. However, the appearance of accurate plasma analyses in the revised NIA-AA AD criteria of 2023 [7] gives hope that blood biomarkers may shortly become a promising screening method for determining the risk of developing AD, as well as, possibly, an early diagnosis tool.

It should be emphasized that saliva is another potential biological substrate used in the diagnosis of neurodegenerative diseases. Numerous studies have addressed the issue of using various biomarkers of neurodegenerative pathologies, including in AD. Similar to our study, the work by Pukhalskaia et al. [28] studied the level of Sirt in saliva. It was found that the levels of Sirt1, Sirt3, and Sirt6 were significantly lower in the group of AD patients compared to the group of healthy individuals, while the levels of Sirt5 did not differ significantly. Our study included patients diagnosed with both AD and POAG, i.e., two neurodegenerative diseases. This may explain the absence of statistical differences in the concentration of Sirt in these groups. It should be noted that evaluation of biomarkers in saliva is still associated with a number of disadvantages, which need to be overcome in the future for saliva biomarkers to be considered potential diagnostic tools. Currently, the literature data in this field remains contradictory.

Our work produced interesting results regarding the correlation between Sirt3 and the ten-words recall test, which evaluates auditory-verbal memory, one of the most important cognitive functions that suffer in AD. Some authors believe that Sirt3 plays a key role in the AD pathogenesis [29]. The current mechanisms of Sirt3 action in AD mainly include an increase in the level of ATP in mitochondria and stimulation of mitochondrial biosynthesis, activating and enhancing mitochondrial dynamics, countering oxidative stress, and regulating neuron excitability. Sirt3 plays a protective role in AD. Animal studies have demonstrated a correlation between a decrease in the concentration of Sirt3 in blood plasma and a decrease in cognitive function in mice [30, 31].

The analysis of MR morphometry data in group 1 of patients showed a statistically significant decrease in the volumes of the right and left hippocampus, right and left entorhinal cortex and cingulate gyrus, as well as the thicknesses of the right and left entorhinal cortex and cingulate gyrus relative to group 2. The results obtained seem reasonable, since these structures are of key importance in the realization of cognitive functions and their disorder is an AD pathognomonic sign. Some publications provide evidence that neuroimaging biomarkers for diagnosis and progression of basal medial temporal structures of the brain have been recently adopted. However, the most vulnerable parts of the medial temporal structures of the brain, which are responsible for the progression of the neurodegenerative process, from the stage of moderate CI to dementia in AD, are atrophy of the hippocampus, entorhinal cortex, and cingulate gyrus [32]. This is consistent with the results of our study. It should be noted that our data on the statistically significant differences between groups 1 and 2 in terms of MR morphometry may demonstrate the progression of the neurodegenerative process and depend on the severity of CI.

In our work, correlations were identified between neuropsychological tests and MR morphometry data. Thus, in AD patients, a decrease in the total score on integrative scales (MMSE, MoCA) correlated with a decrease in the volume of the right and left hippocampus, the volume of the right and left entorhinal cortex, and the thickness of the left cingulate gyrus. In POAG patients, a decrease in the total score on integrative scales (MMSE, MoCA) correlated with a decrease in the volume of the right entorhinal cortex, the volume of left and right cingulate gyri. In both groups, a decrease in the naming of the number of words on the ten-words recall test was associated with a decrease in

the volume of the entorhinal cortex. At the same time, both in group 1 (patients with AD) and group 2 (patients with POAG), a decrease in the blood Aβ42/Aβ40 ratio was associated with a decrease in the volume of the left entorhinal cortex and the volume of the right entorhinal cortex, respectively.

The entorhinal cortex is located in the cortical region adjacent to the hippocampus, occupying most of the parahippocampal gyrus. It plays the role of a connecting link in the information exchange between the associative regions of the neocortex and the hippocampus. It should be noted that the entorhinal cortex forms connections not only with the hippocampus, but also with the cingulate gyrus. The entorhinal cortex, in turn, receives highly processed information from all sensory modalities, contributing to cognitive processes, memory in particular. Recent MRI studies have revealed that the entorhinal cortex is affected primarily in AD [33–34]. Given the established correlation in our two groups between a decrease in the volume of the entorhinal cortex and both a decrease in the Aβ42/Aβ40 ratio and neuropsychological parameters, this neuroimaging parameter can be regarded as a significant early marker of the neurodegenerative process.

CONCLUSION

The results obtained allow us to assume a similar nature of the neurodegenerative process in both AD and POAG. Therefore, POAG may be considered as a predictor of AD development. Considering the above, all patients with POAG may be recommended to undergo a neuropsychological examination in order to diagnose cognitive disorders early and receive timely therapy.

At the same time, the conducted laboratory analysis indicates a relationship between the two neurodegenerative pathologies. Moreover, the study of the Aβ42/Aβ40 ratio in blood plasma seems highly promising. Our findings show that this parameter, unlike Sirt, may reflect the progression of the neurodegenerative process. Research in the direction of searching for AD biomarkers in biological fluids and clarifying their diagnostic accuracy should be continued.

In combination with neuropsychological testing, MR morphometry should be carried out, which allows for a quantitative analysis of the volumes and thicknesses of brain structures, thereby increasing the diagnostic potential of MRI in the early stages of AD and POAG. It should be noted that the statistical differences between the AD and POAG groups based on the results of MR morphometry may be related to the stages of the neurodegenerative process and depend on the CI stage. In these diseases, MR morphometry can be used as a diagnostic screening method for the progression of the neurodegenerative process.

The revealed correlations between neuropsychological parameters and MR morphometry data in patients with AD and POAG once again emphasize the responsibility of the studied brain structures for the implementation of cognitive processes. It is important that neurodegenerative changes based on the results of MR morphometry can be identified at earlier stages of the disease, when CI is minimally different from the norm during neuropsychological testing, as confirmed by our study in patients with POAG. A decrease in the volume and thickness of the entorhinal cortex can be regarded as a significant early marker of the neurodegenerative process.

It can be concluded that a comprehensive assessment of the state of cognitive functions, laboratory and neuroimaging diagnostic methods, as well as diseases associated with the development of AD, such as POAG, represents an important scientific direction. Further studies on larger cohorts of patients are required.

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