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Changes in the activity of lysosomal cysteine proteases of plasma mononuclear and polymorphonuclear blood leukocytes in Alzheimer's disease

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ABSTRACT

Aim. To study the level of activity of lysosomal cysteine proteases (cathepsins H, B, L) in blood plasma and fractionated leukocytes (polymorphonuclear and mononuclear) in patients with Alzheimer's disease in comparison with similar indicators in persons without signs of neurodegeneration as a possible marker of Alzheimer's disease development and diagnosis.

Materials and methods. The spectrofluorimetric study of cathepsins B, L, H activity level in plasma and fractionated leukocytes was conducted in 22 patients diagnosed with Alzheimer's disease in comparison with the same indicators in 22 patients matched by sex, age and associated diseases with patients of the observation group, but having no signs of neurodegeneration.

Results. The activity of all three enzymes, and especially cathepsin H, increased significantly in blood plasma. A significant increase is also noted in the activity of cathepsins H, B, and L in homogenates of fractionated leukocytes. At the same time, in both polymorphonuclear and mononuclear leukocytes the greatest degree of changes is demonstrated by the activity of cathepsin B, and the least is the activity of cathepsin L. Given the available data on an increased cathepsin B activity in the cerebrospinal fluid of patients with Alzheimer's disease, we can assume a correlation between the state of lysosomal proteases activity in the Central nervous system and in the peripheral blood cells.

Conclusion. Alzheimer's disease is associated with increased activity of cysteine cathepsins in plasma, polymorphonuclear and mononuclear leukocytes of peripheral blood, which can be considered as one of the possible markers of development and diagnosis of the disease.

Key words: Alzheimer's disease, neurodegeneration, proteolysis, cysteine cathepsins, blood plasma, polymorphonuclear leukocytes, mononuclear leukocytes.

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Conformity with the principles of ethics. The study complies with ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for the Conducting of Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 266. All patients signed an informed consent to participate in the study. The study protocol was approved by the local ethics committee of Ryazan State Medical University (Protocol No. 6 of 6.11.2018).

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Изменение активности лизосомальных цистеиновых протеаз плазмы, моноядерных и полиморфноядерных лейкоцитов крови при болезни Альцгеймера

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РЕЗЮМЕ

Цель. Изучить уровень активности лизосомальных цистеиновых протеиназ (катепсинов H, B, L) в плазме крови и фракционированных лейкоцитах (полиморфноядерных и моноядерных) пациентов с болезнью Альцгеймера в сравнении с аналогичными показателями у лиц, не имеющих признаков нейродегенерации, как возможный маркер развития и диагностики болезни Альцгеймера.

Материалы и методы. Проведено спектрофлуориметрическое исследование уровня активности катепсинов B, L, H в плазме крови и фракционированных лейкоцитах 22 пациентов с диагнозом «Болезнь Альцгеймера» в сравнении с аналогичными показателями 22 пациентов, сопоставимых по полу, возрасту и сопутствующим заболеваниям с пациентами группы наблюдения, но не имеющих признаков нейродегенерации.

Результаты. В плазме крови статистически значимо повышена активность всех трех ферментов, в наибольшей степени – активности катепсина H. В гомогенатах фракционированных лейкоцитов также отмечается статистически значимое повышение активности катепсинов H, B, L, при этом как в полиморфноядерных, так и в моноядерных лейкоцитах в наибольшей степени изменяется активность катепсина B, наименьшей –катепсина L. Учитывая имеющиеся данные о повышении активности катепсина B в цереброспинальной жидкости пациентов с болезнью Альцгеймера, можно предположить взаимосвязь между состоянием активности лизосомальных протеиназ в центральной нервной системе и периферических клетках крови.

Заключение. Болезнь Альцгеймера ассоциирована с нарастанием активности цистеиновых катепсинов в плазме, полиморфноядерных и моноядерных лейкоцитах периферической крови, что может рассматриваться как один из возможных маркеров развития и диагностики заболевания.

Ключевые слова: болезнь Альцгеймера, нейродегенерация, протеолиз, цистеиновые катепсины, плазма крови, полиморфноядерные лейкоциты, моноядерные лейкоциты.

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INTRODUCTION

Every year, the world's population of elderly people is growing, which leads to an increase in the number of patients with neurodegenerative diseases. Alzheimer's disease (AD) occupies a leading position among them in both prevalence and economic expenses. An important and unresolved problem is the late diagnosis and, accordingly, the late start of treatment, which only begins at the stages of clinically apparent dementia. Therefore, the issue of finding diagnostic methods for AD at the earliest (pre-demented and asymptomatic) stages of the development of the neurodegenerative process, which, according to a number of studies, is 15–20 years ahead of the formation of clinically apparent dementia, remains

relevant. To apply such preventive strategies in relation to AD, it is necessary to search for peripheral biomarkers in environments easily accessible for research (blood serum, saliva, urine). Given the multifactorial nature of neurodegeneration in AD, the creation of a multimodal diagnostic panel is considered more justified [1–3]. One of the possible biomarkers of AD may be a change in the activity of lysosomal enzymes of various cells.

Correct functioning of lysosomes is especially important for neurons, since they cannot reduce the content of accumulated toxic molecules and aggregates by cell destruction [2]. Disruption of lysosomal function plays an important role in the degeneration of neurons and in the pathogenesis of numerous neurodegenerative diseases [4]. In recent years, information indicating the participation of lysosomal proteases in the pathogenesis of AD has appeared [2, 3], although the data are very fragmented and not always unambiguous. However, there is no doubt that proteolytic enzymes are a very sensitive marker of cellular "trouble", and information about the level of activity of these enzymes can be used for early diagnosis and determination of the severity of a number of pathological conditions [5].

It has been proven that the amyloid precursor protein (APP) plays a key role in the pathogenesis of AD. The full-sized APP belongs to the type I transmembrane protein family and is thought to be involved in the regulation of protein transport [6]. The most studied extracellular region consists of several domains: E1, consisting of two subdomains (growth factor-like domain and copper-binding domain), and E2, linked by an acid domain (AcD). APP can undergo various types of proteolytic treatment [4, 5]. Successive cleavage of the protein by alpha and gamma secretase leads to the formation of p3 peptides (non-amyloidogenic pathway). In the case of alternative processing with the help of beta and gamma secretase, a polypeptide consisting of 40–43 amino acid residues (β-amyloid), insoluble in water, aggregating with the formation of polymers deposited in the form of plaques, is formed [7].

APP is the subject of extensive proteolytic treatment; therefore, theories about the effect of lysosomal proteases on the occurrence of AD, the possibility of a diagnostic study of cathepsin activity as a marker, and the use of their inhibitors or inducers in the treatment of the disease have been developed for a long time [8]. Cathepsin B is one of the proteins involved in the regulation of the number of Aβ peptides, but its role in the pathogenesis of AD requires further research. Paradoxically, on the one hand, since it possesses β-secretase activity, it can participate in the formation of Aβ peptides, and on the other hand, it can also participate in the processes of their degradation [6].

It was proved that the cathepsin B sulfhydryl group of cysteine (Cys32) cleaves the Aβ peptide from the carboxyl end at the location of the glutamic acid residue (Glu11), and a decrease in the production and activity of cathepsin B initiates the accumulation of Aβ peptides [9].

It is also known that cysteine cathepsins B and L are involved in the degradation of not only amyloid peptides, but also C-terminal fragments of APP and β-secretase (BACE1), and affect cholesterol metabolism in neurons. A decrease in the activity of these cathepsins or their inhibition leads to lysosomal deficiency, impaired synthesis of NPC1 and ABCA1 proteins that are involved in the release of cholesterol, and impaired degradation of key AD proteins [4].

A recent study showed that cathepsin B can accelerate the metabolism of Aβ peptides via lysosomal pathways and reduce memory deficit associated with AD. Hippocampal injections of the adeno-associated virus expressing cathepsin B decreased Aβ levels, increased Lamp1 and improved learning and memory [10].

At the same time, it is known that pyroglutamate-amyloid-β-peptides (pGlu-Aβ), which are especially harmful forms of amyloid-β-peptides present in the brain in AD, exist. PGlu-Aβ peptides are N-terminal truncated forms of full-sized Aβ peptides in which the N-terminal glutamate is cyclized to pyroglutamate to form pGlu-Aβ (3-40/42). Cathepsin B gene switching off the leads to a decrease in the level of pGlu-Aβ, and the use of an inhibitor of this enzyme (E64d experimental drug) demonstrated a decrease in memory deficit in experimental animals [11].

There is extensive evidence that the accumulation of mononuclear phagocytes, including microglial cells, monocytes and macrophages at the sites of β-amyloid deposition in the brain, is an important pathological characteristic of AD, and the concentration of these cells grouped around Aβ deposits is several times higher than in neighboring brain regions [12]. Since the blood-brain barrier is permeable to mononuclear and polymorphonuclear leukocytes, it can be assumed that changes in the metabolism of these cells may indirectly indicate pathological changes in brain tissue and be a peripheral marker of the neurodegenerative process. The aim of this study is to study the level of activity of lysosomal cysteine proteases (cathepsins H, B, and L) in blood plasma and fractionated leukocytes (polymorphonuclear (PMNL) and mononuclear leukocytes (MNL)) in patients with Alzheimer's disease and to compare with similar indicators in individuals without signs neurodegeneration as a possible marker of Alzheimer's disease development and diagnosis.

MATERIALS AND METHODS

Clinical material for the study were blood plasma and fractionated leukocytes (PMNL and MNL) obtained from 22 patients with Alzheimer's disease who underwent in-patient treatment and dispensary observation at the N.N. Bazhenova Regional Clinical Psychiatric Hospital. All patients included in the observation group have a diagnosis confirmed by instrumental laboratory methods according to modern diagnostic criteria. As a comparison group, we used blood plasma and fractionated leukocytes obtained from 22 patients of the same hospital, comparable in age and gender to patients of the observation group, but who did not have clinical signs of dementia and neurodegeneration. The study complies with ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for the Conducting of Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order of the Ministry of Health of the Russian Federation of June 19, 2003 No. 266.

Blood sampling was performed once on an empty stomach from the ulnar vein in an amount of 10 ml, heparin was used as an anticoagulant. Separation of leukocytes into fractions was carried out by the isopycnic centrifugation method [13]. Counting of leukocytes isolated from samples was carried out in the Goryaev chamber using a R-15 "Biolam" binocular microscope (Russia).

The resulting precipitation of washed leukocytes was brought to a concentration of 106 cells/ml with distilled water containing 0.1% X-100 triton solution and subjected to freezing and thawing three times to destroy plasma and lysosomal membranes. The resulting lysates were used to determine the activity of the studied enzymes [14–16].

The activity of cathepsins L, B, and H was studied by the spectrofluorimetric method of Barrett and Kirschke [17] with the measurement of the fluorescent reaction product 7-amido-4-methylcoumarin, which is formed upon cleavage of specific fluorogenic substrates: Na-carbobenzoxy-L-phenylalanyl-arginine-7-amido-4-methylcoumarin (N-CBZ-Phe-Arg-7-amido-4-methylcoumarin, Sigma, USA) for cathepsin L, arginine-7-amido-4-methylcoumarin (Arg-7-amido-4-methylcoumarin, Sigma, USA) for cathepsin H, and Na-carbobenzoxy-arginine-arginine-7-amido-4-methicoumarin (NaCBZ-Arg-Arg-7-amido-4-methylcoumarin, "Sig ma ", USA).

The activity of cathepsins in blood plasma was calculated in ncat/ml, and the activity of leukocytes in ncat/10^6 cells. For statistical processing of the results, Microsoft Excel and Statistica 10 programs were used. The normality of the distribution of the sample was evaluated by the Shapiro – Wilk test. The groups were compared using the nonparametric Mann – Whitney *U*-test. The result was statistically significant at $p < 0.05$. The results are presented as median, upper and lower quartiles Me (Q_1–Q_3).

RESULTS AND DISCUSSION

In the blood plasma of patients with AD, the activity of cathepsins H, B, L was statistically significantly increased compared to patients who did not have signs of neurodegeneration. Among the studied enzymes, the most significant increase was in the activity of cathepsin H (22-fold increase relative to the comparison group), while the activity of cathepsin B increased 2.8 times, and cathepsin L was 1.9 times (Fig. 1).

In PMNL, the activity of cathepsins B and H was increased 5 and 5.4 times, respectively, and the cathepsin L activity was 2 times (Fig. 2).

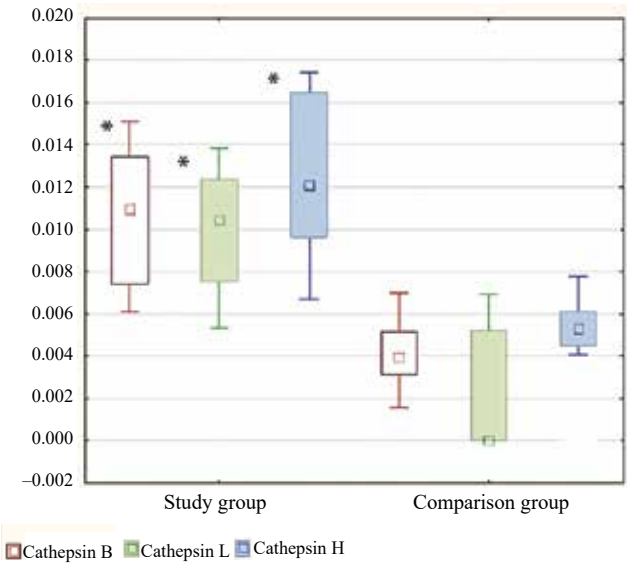

Fig. 1. Activity of plasma cathepsins in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), ncat/ml

* marked statistically significant data.

The following pattern is noted in MNL: the most pronounced change in the cathepsin B activity is a 5 times increase relative to the comparison group, the cathepsin H activity is increased by 3.5 times, and cathepsin L increased by 1.7 times (Fig. 3).

A significant increase in the activity of cathepsin H in the blood plasma of patients with AD is of interest. Cathepsin H is an aminopeptidase with endopeptidase activity. It is possible that the increase in activity is relative, since the decrease in the activity of cathepsins B and L correlates with the accumulation of Aβ [10].

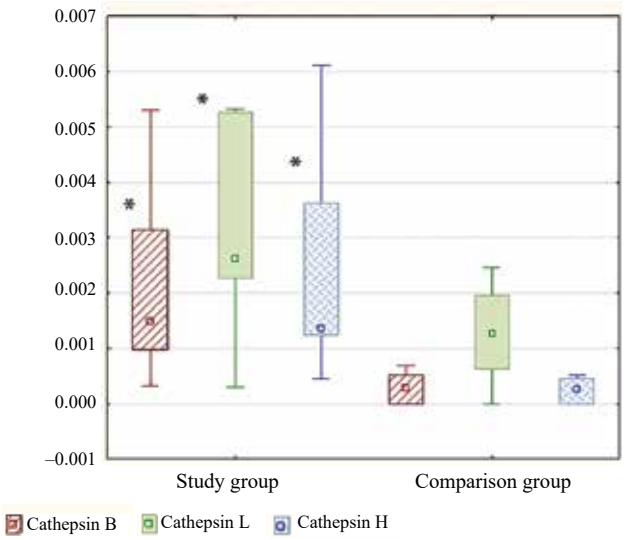

Fig. 2. Activity of polymorphonuclear leukocyte cathepsins in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), nkat/10^6 cells

* marked statistically significant data

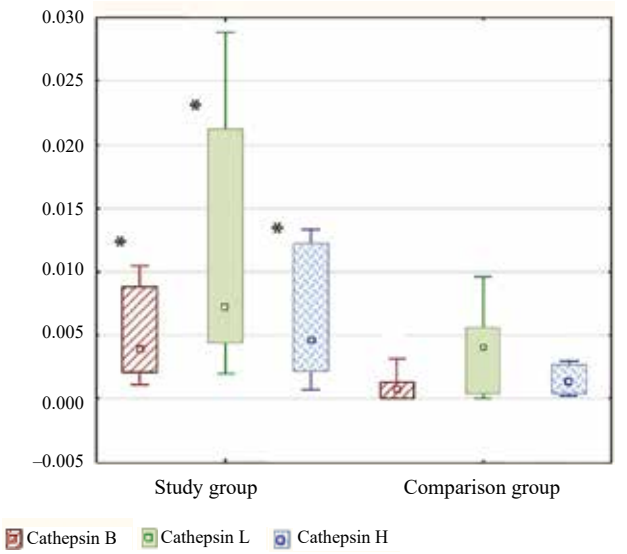

Fig. 3. Activity of cathepsin mononuclear leukocytes in patients with Alzheimer's disease (observation group) and patients without signs of neurodegeneration (comparison group), nkat/10^6 cells

* marked statistically significant data

However, on the other hand, an increase in the activity of cathepsin H can be explained by the participation of this enzyme in the metabolism of modified low-density lipoproteins, which include aprotein E (ApoE). The participation of ApoE in both the formation of amyloid plaques and the metabolism of APP has been proven. The ApoE4 isoform is the most susceptible to protease cleavage, and the resulting C-terminal fragment of the molecule has pronounced neurotoxic properties [6]. The increased activity of cathepsin B can be explained by its ability to penetrate the blood-brain barrier [8], as well as the active participation of this enzyme in the metabolism of APP.

The obtained results suggest the involvement of lysosomal leukocyte proteases in the neurodegenerative process, which is consistent with literature data on the participation of these blood cells in this pathology.

In fractionated leukocytes, the following unidirectional tendency is observed: a predominant increase in the cathepsins B and H activity against the background of a slight increase in the cathepsin L activity. Specific neutrophil granules contain more than 20 different types of proteases, and a huge number of receptors that determine their functional activity (for various interleukins, complement system factors and other biologically active molecules) are located on cell membranes. PMNLs contain a large number of lysosomes; various factors, including hypoxia, oxidative stress, and decreased insulin synthesis, can lead to a violation of the integrity of the lysosomal membrane [18]. Numerous studies in recent years prove the correlation between insulin resistance in brain tissue and neurodegenerative processes [19]. The destruction of PMNL lysosomal membranes and the release of cathepsins B and H into the cytoplasm can be considered a pathogenetically significant factor in AD and the development of neurodegeneration. Given the increased concentration of these cells around Aβ and the ability of leukocytes to penetrate the blood-brain barrier, a change in the activity level of blood leukocyte cathepsins is an important biomarker of the neurodegenerative process.

In literature there are references to an increase in the cathepsin B activity in the cerebrospinal fluid of patients with AD [20], which suggests a correlation between the state of lysosomal cysteine proteases activity in the central nervous system and peripheral blood cells. This means the studied parameter can be considered as a possible marker for the AD diagnosis.

CONCLUSION

Alzheimer's disease is associated with increased activity of plasma cysteine cathepsins, and polymorphonuclear and mononuclear peripheral blood leukocytes, which can be considered as one of the possible markers of the development and diagnosis of the disease.

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