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ANALYSIS OF THE EXPRESSION LEVEL OF CIRCULATING MICRORNAS IN THE BLOOD OF PATIENTS WITH ALZHEIMER'S DISEASE

Over the past few decades, there has been a steady decline in fertility worldwide, while life expectancy has been increasing. This is leading to an ageing population. In today's aging world, cognitive disorders and dementia in the elderly are key problems. Thus studying the regulation of the molecular mechanisms of age-related cognitive impairment is an urgent task. Fourteen elderly and senile individuals with Alzheimer's disease participated in this study. The expression of circulating microRNAs at all stages was determined using kits from Qiagen (Germany).

A study was conducted to determine the expression level of circulating microRNAs: hsa-mir-483, hsa-miR-132, hsa-mir-29c, hsa-mir-193b in the blood serum of elderly and senile people suffering from Alzheimer's disease.

The data obtained by us indicates that in patients suffering from Alzheimer's disease, the levels of these micro-RNAs depended on age and the degree of cognitive impairment. Circulating mir-132-5p microRNA was detected in the blood serum of senile people suffering from moderate dementia, in contrast to elderly people with mild degree of dementia.

Keywords: Alzheimer's disease, cognitive impairment, circulating microRNAs, β-amyloid, tau protein.

Introduction. Over the past few decades, there has been a steady decline in fertility worldwide, while life expectancy has been increasing. This leads to an aging population, which is becoming a global phenomenon and perhaps one of the most significant social changes of the 21st century. In today's aging world, cognitive disorders and dementia in the elderly are key issues.

In Russia, the population of patients with Alzheimer's disease is 1 million 248 thousand people. However, less than 10% of the estimated number of patients with dementia has been officially registered [2, 3].

Alzheimer's disease is a neurodegenerative disease characterized by a gradual, barely noticeable onset in pre-senile or elderly age, a steady progression of memory and higher brain functions disorders leading to dementia, with the formation of a characteristic complex of neuropathological, neuroimaging and biochemical signs [2].

Many risk factors lead to the development of Alzheimer's disease, which are conditionally divided into modifiable and unmodifiable. The risk of developing Alzheimer's disease increases in the presence of risk factors such as low intellectual activity, physical inactivity, obesity, smoking, uncontrolled hypertension, hyperlipidemia, diabetes mellitus, etc. [1]. Elderly and senile age, a family history of Alzheimer's disease and the carriage of genetic polymorphisms, the presence of the e4 allele of apolipoprotein E, female gender, and a history of traumatic brain injuries are among the unmodifiable risk factors for the development of this disease.

The current hypothesis of the development of the disease suggests that β-amyloid or amyloid plaques initiate a pathophysiological cascade leading to the accumulation of intracellular tau protein, which spreads through the cerebral cortex, directly triggering the process of neurodegeneration and the development of clinical manifestations of Alzheimer's disease [5].

Currently, an active search is underway for effective markers of the molecular mechanism of disease development. Research in recent decades has clearly demonstrated the important role of microRNAs in the development of the pathogenesis of Alzheimer's disease through post-transcriptional control of gene expression.

The aim of the study was to determine the expression level of circulating microRNAs: hsa-mir-483, hsa-miR-132, hsa-mir-29c, hsa-mir-193b in the blood serum of elderly and senile people suffering from Alzheimer's disease.

Materials and methods of research. This study was conducted at the Geriatric Center of the Republican Clinical Hospital No. 3 (RCH No. 3) in Yakutsk. 14 people with Alzheimer's disease were randomly selected (Table 1). The diagnosis was es-Tableished in accordance with the clinical recommendations "Cognitive disorders in the elderly and senile" approved by the Ministry of Health of the Russian Federation in 2020 [2]. The study was approved by the local Ethics Committee of the M.K. Ammosov Northeastern Federal University. Voluntary informed consent to participate in the study was obtained from each patient and/or guardians/relatives.

In the study, patients were divided into two age groups, age correlated with the degree of cognitive impairment. Venous blood sampling was performed in the morning, on an empty stomach. To obtain the serum, the blood was centrifuged at 3000 rpm for 7 minutes (4 °C). Biological samples were frozen and stored at a temperature of -85 ^{0} C. before the study.

Before the start of total RNA isolation, plasma was purified from cellular debris,

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apoptotic cells and blood platelets by double centrifugation: the first - 800 rpm, the second - 12,000 rpm.

The expression level of the following microRNAs was determined in the blood serum of all examined patients: hsa-mir-483, hsa-miR-132, hsa-mir-29c, hsa-mir-193b. To quantify microRNAs in real time, in accordance with the recommendations of Qiagen, the authors developed primers (Table 2). The sequences of microRNA primers were taken from the microRNA database: https://www.mirbase.org.

Total RNA extraction was performed by the trizol-chloroform method. Total RNA was subjected to reverse transcription according to the protocol of the manufacturer of the miRCURY LNA RT Kit (art. 339340, "Qiagen"). After that, real-time PCR was performed using the miRCURY LNA SYBR Green PCR kit 200 (art. 339345, "Qiagen"). Reverse transcription and polymerase chain reaction were performed on a BioRad CFX96 device (Bio-Rad, USA).

Exogenous microRNA - cel-miR-39-3p belonging to Caenorhabditis elegans (RNA Spike-In Kit, For RT and miRCURY LNA miRNA PCR Assay art. 339390 and YP00203952, respectively) was used as a control for the samples, with respect to which the concentration of the studied microRNAs were derived. The microRNA expression levels were calculated using the ΔCt method as follows: ΔCt = average Ct value References microRNA (cel-miR-39-3p) - average Ct value (microRNA of interest). The level of relative microRNA expression corresponded to the value of 2^ (ΔCt).

Statistical analysis was performed using the SPSS 18.0 for Windows program ("SPSS, Inc.", Chicago, IL, USA). The differences between the groups were assessed using the Mann-Whitney U-test. The comparison of the indicators was carried out using the chi-square test. Correlations were determined using Spearman's rank correlation. A statistically significant difference was considered to be the value of $p<0.05$.

Results and discussion. Among all the microRNAs studied by us, statistically significant differences between elderly and senile individuals were observed in the content of mir-132. mir-132 was not detected in the blood of elderly people, while in senile people the expression level of circulating microRNA reached 0.035±0.002 cu (Figure).

The levels of circulating mir-193b in the blood of elderly and senile people did not change statistically, but we discovered that the content of mir-193b in the blood of senile people tended to decrease.

The level of circulating mir-29c and mir-483 tended to increase in the group of elderly people, but did not reach statistic significance.

We analyzed academic sources on the nature of changes in the level of microRNAs in patients suffering from moderate cognitive impairment and Alzheimer's disease (Table 3).

Many studies have shown that the level of circulating miR-132 increases in the blood in neurodegenerative diseases such as Alzheimer's disease [26], Parkinson's disease [27], multiple sclerosis [8] and amyotrophic lateral sclerosis [21], this fact emphasizes its connection with

Table 1

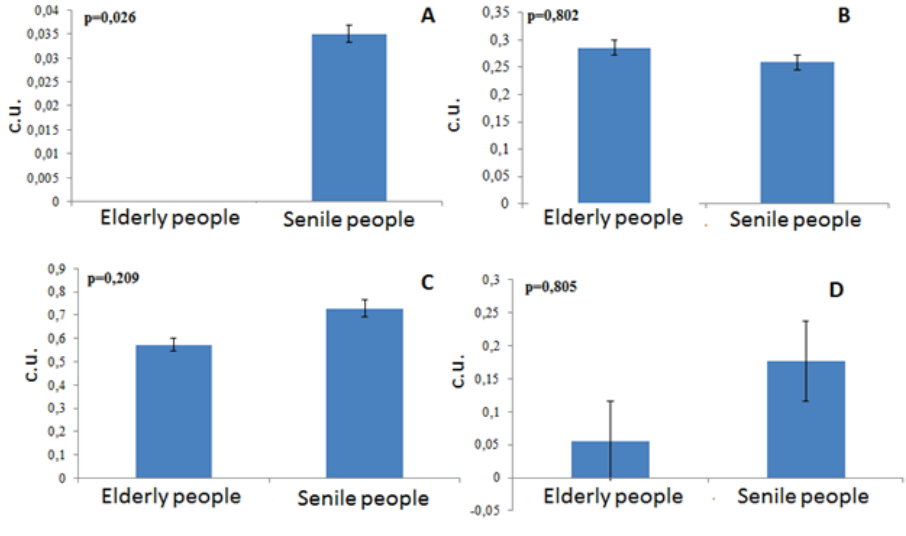
Brief description of the patients included in the study

Research Group	Elderly patients	Senile patients
Number of patients, n	7	7
Sex, n	Male - 4 Female - 3	Male - 5 Female - 2
Age at the moment of research, years	61.71±10.35	82.14±2.76
Concomitant diseases, n	Angina pectoris - 1 Hypertension - 6	Hypertension - 1 Angina pectoris - 2 Encephalopathy - 4
The stage of development of Alzheimer's disease according to the Clinical Dementia Rating (CDR) scale, n	1 point - 6 2 points - 1	2 points - 7
Bartel index, n	100 points - 1 96 points - 6	61 points - 7

Table 2

Sequences of primers used in the study

microRNAs	Праймеры
hsa-miR-483-5p	*5'- AAG ACG GGA GGA AAG AAG GGA-3'*
hsa-miR-132-5p	*5'- ACC GTG GCT TTC GAT TGT TAC TAA A -3'*
hsa-miR-29c-5p	*5'- GAC CGA TTT CTC CTG GTG TTC -3'*
hsa-miR-193b-5p	*5'- GGG TTT TGA GGG CGA GAT GAA -3'*



Expression levels of circulating microRNAs: mir-132 (A), mir-193b (B), mir-29c (C), mir-483 (D) in the blood serum of patients with Alzheimer's disease

Table 3

A brief review of studies on the expression of microRNAs (mir-29c, mir-193b, mir-483) in patients with cognitive impairment

microRNAs	Number of examined	Sample	Difference	Sources
mir-29c	AD (n = 20), Ctrl (n = 20)	Serum	↓	[24]
mir-193b	MCI (n = 43), AD (n = 51)	Plasma	↓	[13]
mir-483	AD (n = 20), MCI (n = 20), Ctrl (n = 20)	Plasma	↑	[15]
	AD (n = 20), MCI (n = 34), Ctrl (n = 37)	Plasma	↑	[14]

Note. AD - Alzheimer's disease; Ctrl – individuals in the control group; MCI-moderate cognitive impairment.

Table 4

Predicted/confirmed target genes of hsa-mir-132-3p involved in the pathogenesis of Alzheimer's disease

microRNA	Gene	Transcript binding site in an untranslated area	Cumulative PCT
mir-132	*MAPT*	4113-4119	0.5
	PTBP2	57-63	0.51
	PTBP2	372-378	< 0.1
	PTBP2	4506-4512	< 0.1
	SIRT1	1680-1686	0.38
	SIRT1	1614-1620	< 0.1
	MAPK1	1379-1386	0.78
	MAPK1	2225-2232	0.77
	MAPK1	8111-8118	< 0.1

neuropathological processes and determines its potential as a biomarker of neurodegenerative diseases.

It should be noted that human mir-132 consists of two homologous microRNAs: hsa-mir-132-5p and hsa-mir-132-3p. Mir-132 is evolutionarily conservative and has the same sequence and structure in humans, rats, mice, monkeys and other species. Mir-132 has tissue specificity and is highly expressed in nerve-related tissues [28].

To determine the molecular mechanisms by which mir-132-5p may be involved in the development of Alzheimer's disease, we used the TargetScan Release 7.1 database to predict mir-132-5p binding targets (Table 4). Analysis using the TargetScan database showed that mir-132 directly targets gene transcripts: *MAPT* (Tau protein) and *PTBP2* (protein 2 binding the polypyrimidine tract). This suggests that an increase in the level of mir-132 may have protective properties, since it reduces the amount of tau protein, however, overexpression of this microRNA changes the ratio of tau protein isomers 4R:3R in neuronal cells, which can lead to the development of neurodegenerative diseases [6].

According to the TargetScan database, in the human body, mir-132 suppresses the expression of three gene transcripts: *SIRT1* (sirtuin-1 deacetylase) and *MAPK1* (mitogen-activated protein kinase). The protein expressed by the *SIRT1* gene has protective properties: it protects the brain of mice from neurodegenerative diseases [12], and also demonstrates the phenotype of delayed aging and an increase in life expectancy [16]. The results obtained by Hadar et al. practically confirm the involvement of mir-132 in the regulation of transcript expression of the *SIRT1* gene [11]. It has been esTableished that in Alzheimer's patients, the MAPK1 enzyme *is* activated at an early stage [19], which indicates the involvement of this enzyme in the pathological process. In a study by Deng et al. Activation of mir-132 expression has been shown to improve the cognitive functions of rats with Alzheimer's disease by inhibiting the MAPK1 signaling pathway [7].

In addition, a study by Wang et al. has shown that a decrease in the level of mir-132 leads to an increase in the amount of the nitric oxide synthase-1 (NOS1) enzyme and triggers excessive production of nitric oxide followed by aberrant S-nitrosylation (SNO) of specific proteins associated with neurodegeneration and tau pathology, such as cyclin-dependent kinase-5. This leads to an increase in tau protein phosphorylation and the development of neurodegenerative diseases [23].

Walgrave et al. has shown that the pathogenesis of Alzheimer's syndrome leads to a deficiency of mir-132 in mouse brain tissue, and the addition of mir-132 alleviates memory deficiency in Alzheimer's disease [22]. Smith et al. and Xie et al. have found that mir-132 deficiency in the mouse brain leads to increased tau protein expression, phosphorylation and aggregation in mice [18, 25].

Thus, the data obtained by us indicate that in the body of patients suffering from Alzheimer's disease, the levels of micro-RNA: hsa-mir-483, hsa-mir-132, hsa-mir-29c, hsa-mir-193b depended on age and degree of cognitive impairment. Circulating mir-132-5p microRNA was detected in the blood serum of senile people suffering from moderate dementia, in contrast to elderly people with mild degree of dementia.

References

1. Gromova D.O. Novoe v terapii bolezni Al'cgejmera [New era of treatment of Alzheimer's disease]. Povedencheskaya nevrologiya [Behavioral Neurology. 2021; 2: 48–55 (In Russ.).] DOI 10.46393/2712-9675_2021_2_48_55

2. Klinicheskie rekomendacii. Kognitivnye rasstrojstva u lic pozhilogo i starcheskogo vozrasta. Utverzhdeny Minzdravom RF v 2020 godu. Okonchanie dejstviya: 2022 god. ID: 617. Razrabotany: Obshchestvennoj organizaciej "Rossijskoe obshchestvo psihiatrov", Obshcherossijskoj obshchestvennoj organizaciej «Rossijskaya associaciya gerontologov i geriatrov». Odobreny Nauchno-prakticheskim Sovetom Minzdrava RF [Clinical recommendations. Cognitive disorders in elderly and senile people. Approved by the Ministry of Health of the Russian Federation in 2020. Expiration date: 2022. ID: 617. Developed by: the public organization "Russian Society of Psychiatrists", the All-Russian public organization "Russian Association of Gerontologists and Geriatricians". Approved by the Scientific and Practical Council of the Ministry of Health of the Russian Federation. 170 p. (In Russ.).]

3. Lobzin V.Yu., Kolmakova K.A., Emelin. A.Yu. Novyj vzglyad na patogenez bolezni Al'cgejmera: sovremennye predstavleniya o klirense amiloida [A new look at the pathogenesis of Alzheimer's disease: modern ideas about amyloid clearance]. Obozrenie psihiatrii i medicinskoj psihologii im. V.M. Bekhtereva [Review of psychiatry and medical psychology named after V.M. Bekhterev. 2018; (2): 22-28 (In Russ.).]

4. Lokshina A.B. Tyazhelaya demenciya: diagnostika, vedenie pacientov, profilaktika oslozhnenij [Severe dementia: diagnosis, patient management, prevention of complications]. Nevrologiya, nejropsihiatriya, psihosomatika [Neurology, neuropsychiatry, psychosomatics. 2014; (1): 54–60 (In Russ.).] DOI: tp://dx.doi.org/10.14412/2074-2711-2014-1-54-60.

5. Tappakhov A.A. [et. al.]. Trudnosti diagnostiki atipichnyh variantov bolezni Al'cgejmera [Difficulties in diagnosing atypical variants of Alzheimer's disease]. Rossijskij nevrologicheskij zhurnal [Russian neurological journal. 2021; 26(5): 16-23 (In Russ.).]

6. Capano LS, Sato C, Ficulle E, et al. Recapitulation of endogenous 4R tau expression and formation of insoluble tau in directly reprogrammed human neurons. Cell Stem Cell. 2022; 29(6):918-932.e8. doi:10.1016/j.stem.2022.04.018

7. Deng Y, Zhang J, Sun X, et al. miR-132 improves the cognitive function of rats with Alzheimer's disease by inhibiting the MAPK1 signal pathway. Exp Ther Med. 2020; 20(6):159. doi:10.3892/etm.2020.9288

8. Dolati, S., Aghebati-Maleki, L., Ahmadi, M., Marofi, F., Babaloo, Z., Ayramloo, H., Jafarisavari, Z., Oskouei, H., Afkham, A., Younesi, V., Nouri, M., & Yousefi. Nanocurcumin restores aberrant miRNA expression profile in multiple sclerosis, randomized, double-blind, placebo-controlled trial. Journal of cellular physiology. 2018; 233(7): 5222–5230. https://doi.org/10.1002/jcp.26301

9. Frisoni GB, Altomare D, Thal DR, et al. The probabilistic model of Alzheimer disease: the amyloid hypothesis revised. Nat Rev Neurosci. 2022; 23(1): 53-66. doi:10.1038/s41583-021-00533-w

10. Ghetti B, Oblak AL, Boeve BF, Johnson KA, Dickerson BC, Goedert M. Invited review: Frontotemporal dementia caused by microtubule-associated protein tau gene (MAPT) mutations: a chameleon for neuropathology and neuroimaging [published correction appears in Neuropathol Appl Neurobiol. 2015 Jun;41(4):571]. Neuropathol Appl Neurobiol. 2015;41(1):24-46. doi:10.1111/nan.12213

11. Hadar A, Milanesi E, Walczak M, et al. SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's Disease. Sci Rep. 2018;8(1):8465. Published 2018 May 31. doi:10.1038/s41598-018-26547-6

12. Kim D, Nguyen MD, Dobbin MM, et al. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. EMBO J. 2007;26(13):3169-3179. doi:10.1038/sj.emboj.7601758

13. Liu CG, Song J, Zhang YQ, Wang PC. MicroRNA-193b is a regulator of amyloid precursor protein in the blood and cerebrospinal fluid derived exosomal microRNA-193b is a biomarker of Alzheimer's disease. Mol Med Rep. 2014;10(5):2395-2400. doi:10.3892/mmr.2014.2484

14. Nagaraj S, Laskowska-Kaszub K, Dębski KJ, et al. Profile of 6 microRNA in blood plasma distinguish early stage Alzheimer's disease patients from non-demented subjects. Oncotarget. 2017;8(10):16122-16143. doi:10.18632/oncotarget.15109

15. Sabry R, El Sharkawy RE, Gad NM. MiRNA -483-5p as a Potential Noninvasive Biomarker for Early Detection of Alzheimer's Disease. Egypt J Immunol. 2020;27(2):59-72. https://www.researchgate.net/publication/355170638_MiRNA_-483

16. Satoh A, Brace CS, Rensing N, et al. Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab. 2013;18(3):416-430. doi:10.1016/j.cmet.2013.07.013

17. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. Lancet. 2021;397(10284):1577-1590. doi:10.1016/S0140-6736(20)32205-4

18. Smith PY, Hernandez-Rapp J, Jolivette F, et al. miR-132/212 deficiency impairs tau metabolism and promotes pathological aggregation in vivo. Hum Mol Genet. 2015;24(23):6721-6735. doi:10.1093/hmg/ddv377

19. Sun A, Liu M, Nguyen XV, Bing G. P38 MAP kinase is activated at early stages in Alzheimer's disease brain. Exp Neurol. 2003;183(2):394-405. doi:10.1016/s0014-4886(03)00180-8

20. van der Kant R, Goldstein LSB, Ossenkoppele R. Amyloid-β-independent regulators of tau pathology in Alzheimer disease. Nat Rev Neurosci. 2020;21(1):21-35. doi:10.1038/s41583-019-0240-3

21. Vrabec, K., Boštjančič, E., Koritnik, B., Leonardis, L., Dolenc Grošelj, L., Zidar, J., Rogelj, B., Glavač, D., & Ravnik-Glavač, M. (2018). Differential Expression of Several miRNAs and the Host Genes AATK and DNM2 in Leukocytes of Sporadic ALS Patients. Frontiers in molecular neuroscience, 11, 106. https://doi.org/10.3389/fnmol.2018.00106

22. Walgrave H, Balusu S, Snoeck S, et al. Restoring miR-132 expression rescues adult hippocampal neurogenesis and memory deficits in Alzheimer's disease. Cell Stem Cell. 2021;28(10):1805-1821.e8. doi:10.1016/j.stem.2021.05.001

23. Wang Y, Veremeyko T, Wong AH, et al. Downregulation of miR-132/212 impairs S-nitrosylation balance and induces tau phosphorylation in Alzheimer's disease. Neurobiol Aging. 2017;51:156-166. doi:10.1016/j.neurobiolaging.2016.12.015

24. Wu Y, Xu J, Xu J, et al. Lower Serum Levels of miR-29c-3p and miR-19b-3p as Biomarkers for Alzheimer's Disease. Tohoku J Exp Med. 2017;242(2):129-136. doi:10.1620/tjem.242.129

25. Xie AJ, Hou TY, Xiong W, et al. Tau overexpression impairs neuronal endocytosis by decreasing the GTPase dynamin 1 through the miR-132/MeCP2 pathway. Aging Cell. 2019;18(3):e12929. doi:10.1111/acel.12929

26. Xie, B., Zhou, H., Zhang, R., Song, M., Yu, L., Wang, L., Liu, Z., Zhang, Q., Cui, D., Wang, X., & Xu, S. (2015). Serum miR-206 and miR-132 as Potential Circulating Biomarkers for Mild Cognitive Impairment. Journal of Alzheimer's disease : JAD, 45(3), 721–731. https://doi.org/10.3233/JAD-142847

27. Yang, Z., Li, T., Li, S., Wei, M., Qi, H., Shen, B., Chang, R. C., Le, W., & Piao, F. (2019). Altered Expression Levels of MicroRNA-132 and Nurr1 in Peripheral Blood of Parkinson's Disease: Potential Disease Biomarkers. ACS chemical neuroscience, 10(5), 2243–2249. https://doi.org/10.1021/acschemneuro.8b00460

28. Zhang, M., & Bian, Z. (2021). Alzheimer's Disease and microRNA-132: A Widespread Pathological Factor and Potential Therapeutic Target. Frontiers in neuroscience, 15, 687973. https://doi.org/10.3389/fnins.2021.687973