Herpesvirus infections and immunological disturbances in patients with different stages of Alzheimer's disease

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Introduction. Alzheimer's disease (AD) is a multifactorial disease that leads to a progressive memory loss, visual-spatial impairments, emotional and personality changes. As its earliest pre-dementia clinical stage, amnestic mild cognitive impairment syndrome (aMCI) is currently considered. Neuroinflammation plays a role in the development and progression of aMCI and the initial stage of AD, which can be supported by immunological disorders of a systemic character. Study of factors, including infections, influencing immune disorders and systemic inflammatory response in patients with aMCI, is of great importance.
The **aim** of this study was to obtain new data on the possible role of herpesvirus infections in the development and progression of aMCI.
Material and methods. 100 patients with aMCI diagnosis, 45 patients with AD, 40 people from the control group were enrolled into the study. The frequency of DNA detection of herpesviruses (Epstein–Barr virus (EBV), human herpesviruses (HHV) type 6 and 7, cytomegalovirus (CMV)), the levels of viral load and the serological markers of herpesvirus infections (IgG to HHV-1, IgG to CMV) were determined. Immunological studies included an assessment of the level of the main pro-inflammatory and anti-inflammatory cytokines, and indicators of humoral and cellular immunity.
Results. The study found an increased detection rate of EBV in saliva and a higher level of EBV DNA in saliva in aMCI and AD than in the control group. A relationship between the presence of active EBV infection and changes in immunological parameters in patients with aMCI were found. It was also discovered that the level of IgG antibodies to CMV is associated with the stage of AD.
Discussion. The results indicate a possible role of EBV- and CMV-induced infections in the development of immunological changes which are typical for mild cognitive impairment and in the progression of AD.
Conclusion. The obtained data can be important for prognostic methods addressing AD development, including its pre-dementia stage, and for new approaches to individualized treatment and prevention.

Key words: *Alzheimer's disease; amnestic mild cognitive impairment; inflammation; herpesviruses; Epstein–Barr virus*

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Герпесвирусные инфекции и иммунологические нарушения при различных стадиях когнитивных расстройств альцгеймеровского типа

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Введение. Болезнь Альцгеймера (БА) – мультифакториальное заболевание, ведущее к прогрессирующему снижению памяти, зрительно-пространственных функций, эмоциональным и личностным изменениям. В настоящее время в качестве его наиболее раннего додементного клинического этапа рассматривается синдром мягкого когнитивного снижения амнестического типа (amnestic mild cognitive impairment, aMCI). В развитии и прогрессировании aMCI и начальной стадии БА играет роль нейровоспаление, которое может поддерживаться иммунологическими нарушениями системного характера. С учётом этого представляется актуальным исследование факторов (включая инфекционные), влияющих на характер иммунного статуса и выраженность системного воспалительного ответа у страдающих когнитивными расстройствами альцгеймеровского типа на различных стадиях.
Цель данной работы – получение новых данных о возможной роли герпесвирусов в возникновении и прогрессии aMCI и БА.
Материал и методы. Обследованы 100 больных с диагнозом aMCI, 45 пациентов с БА и 40 лиц контрольной группы. Определяли частоту выявления ДНК герпесвирусов (вирус Эпштейна–Барр (EBV), герпесвирусы человека 6 и 7 типов (HHV-6, HHV-7), цитомегаловирус (CMV)), уровни вирусной нагрузки, серологические маркёры герпесвирусных инфекций (ГВИ) (HHV-1- и CMV-инфекции). Иммунологические исследования включали оценку концентрации основных про- и противовоспалительных цитокинов, показателей гуморального и клеточного иммунитета.
Результаты. Установлены повышенная частота обнаружения EBV в слюне и более высокие уровни ДНК EBV в слюне при когнитивных расстройствах альцгеймеровского типа по сравнению с контрольной группой. Выявлено наличие связи между присутствием активной EBV-инфекции и изменениями иммунологических показателей у лиц с aMCI. Обнаружено, что уровень антител (АТ) IgG к CMV связан со стадией когнитивных расстройств у больных.
Обсуждение. Результаты указывают на возможную роль ГВИ, вызываемых EBV и CMV, в развитии иммунологических изменений при мягком когнитивном снижении и прогрессировании когнитивных расстройств альцгеймеровского типа.
Заключение. Полученные данные могут иметь значение для разработки методов прогнозирования течения БА, в том числе на её додементной стадии, и подходов к индивидуализированной терапии и профилактике.

Ключевые слова: *болезнь Альцгеймера; мягкое когнитивное снижение амнестического типа; воспаление; герпесвирусы; вирус Эпштейна–Барр*

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Introduction

Alzheimer's disease (AD) is a multifactorial disorder leading to progressive memory loss, visual-spatial impairments, behavioral and personality changes. Most people develop its symptoms at the age of >65 years. AD-related dementia is seen as the final stage of long-term progressive neurodegenerative brain damage; there are currently no effective treatments. The preclinical stage can last up to 25 years and is characterized by neuronal cell death at faster rates than during the normal aging process. AD symptoms become apparent after 75–85% of the total cell number is lost. Approximately half of patients tend to develop a syndrome of amnestic mild cognitive impairment (aMCI) prior to dementia. They tend to remain independent in their daily living activities and are able to perform professional functions; however, the patients and/or their family and environment notice decline in cognitive abilities, which is confirmed by neuropsychological tests. Patients with aMCI progress to dementia at a rate of 10 to 15% per year [1]. These data emphasize the importance of its prevention in such patients: The therapy that would delay the AD development for another 5 years and, therefore, would help reduce the number of individuals affected by dementia by 2.5 million in the United States alone [2]. Besides, development of prognostic methods addressing cognitive disorders underlying aMCIs is of critical importance. Therefore, a great deal of attention is given to studying of the pathogenesis of this syndrome, including identification of exogenous and endogenous factors that may affect its progression [2].

Earlier, we and other researchers found the relationship of levels of systemic inflammatory markers and humoral immunity activation with the severity of Alzheimer-type cognitive changes and the risk of aMCI progression [3–5]. Exploration of the factors that cause immune disorders and systemic inflammatory response is an increasingly important area of aMCI research. There is a hypothesis suggesting that infections caused by opportunistic pathogens, including herpesviruses, may play a significant role in development and maintaining of the inflammatory response during the latent period of neurodegenerative diseases [6–11]. The representatives of this group, including herpes simplex virus type 1 or human herpesvirus 1 (HSV-1 or HHV-1), human herpesvirus 6 (HHV-6), Epstein–Barr virus (EBV), cytomegalovirus (CMV), are characterized by tropism targeting nervous tissue and are able to invade the brain. Some studies show that they are being found in AD patients' brain tissue with increasing frequency [12, 13]. Even remaining latent, these infectious agents activate microglial cells (macrophages or brain immune cells), which release pro-inflammatory cytokines as well as other mediators and cause oxidative stress, damage and inflammatory response [14–16]. In the meantime, no comparative studies have been performed so far to assess the detection frequency for herpesviruses and viral loads in saliva in patients with different severity levels of AD and aMCI. Little attention has been given to the potential relationship between herpes virus infection (HVI) and clinical progression of Alzheimer-type cognitive disorders.

This work was aimed to obtain new data on a potential role of herpesviruses in development and progression of cognitive disorders. The study objectives included analysis of HVI markers in groups of aMCI and AD patients, assessment of relationships between the viral infections, the nature and extent of immune changes, and the stage of the disease. For the above purpose, we estimated the DNA detection frequency for herpesviruses (EBV, CMV, human herpesvirus 6 and 7 (HHV-6, HHV-7)), viral loads, HVI serological markers (for HHV-1 and CMV infections). The immunology research included assessment of levels of major pro- and anti-inflammatory cytokines as well as indicators of humoral and cell-mediated immunity.

Material and methods

One of the study groups was composed of 100 patients diagnosed with aMCI (aged 54–84 years; mean age 72.6 ± 4.6 years), 45 patients with AD (aged 64–86 years; mean age 74.3 ± 5.7 years; out of them 17 with mild, 12 with moderate, and 16 with severe dementia). The control group included 40 individuals without cognitive disorders and comparable with the main group in terms of age and sex. The inclusion criteria were the ability of a patient to sign and date the informed consent or the presence of the patient's representative authorized to perform the above actions; the age ≥40 years; the AD diagnosis in compliance with criteria adopted by NINCDS/ADRDA (National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association) and the score of ≤26 points based on the Mini-Mental State Examination (MMSE) or the

aMCI diagnosis in accordance with the operational criteria combined with the MMSE score of ≥27 points; the Hachinski scale-based score of ≤4 points; the Geriatric Depression Scale score of ≤10 points. Patients did not have any acute infectious diseases, acute inflammation of the oral cavity or oral mucosa injuries. The participants and their relatives read the study protocol and signed the informed consent. The study was approved by the Ethics Committee of the FSBSI «Mental Health Research Center». All the enrollees underwent general clinical examination, had their cognitive functions assessed against the neuropsychological scales, and had their saliva and blood samples collected.

The enzyme-linked immunosorbent assay (ELISA) was performed to measure cytokine concentrations (interleukins IL-1β, IL-8, IL-10, tumor necrosis factor alpha, TNF-α) in the patients' blood serum (Cytokine LLC, Russia), C-reactive protein as acute phase marker (Khema LLC, Russia), circulating immune components (Khema LLC), total immunoglobulin G (IgG) (Khema LLC), IgG-class antibodies (ABs) for HHV-1 (Bender MedSystems, Austria) and CMV (RADIM, Italy). To analyze the variables of cell-mediated immunity, we used the multicolor flow cytometry technique (monoclonal ABs for immunophenotyping (Becton Dickinson, USA) to differentiate CD45, CD3, CD4, CD8, CD19, CD16, CD56, HLA-DR antigens.

The DNA of lymphotropic herpesviruses: HHV6, HHV7, EBV, and CMV – were identified and quantified by using a real-time polymerase chain reaction (PCR) with fluorescent dye-labelled probes. We used an AmpliPrime RIBO-prep reagent kit to extract nucleic acids from the clinical material (AmpliSens, Russia), hemolytic reagent (AmpliSens), an AmpliSens EBV/CMV/HHV6-screen-FL kit (AmpliSens) to identify and quantify EBV, CMV, and HHV-6 DNAs in the clinical material, and the AmpliSens HHV7-screen/monitor-FL (AmpliSens), a similar kit of reagents for detection/quantification of the HHV-7 DNA. These reagent kits were designed at the FSBI «Central Research Institute for Epidemiology» of Rospotrebnadzor (the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing) in compliance with the TU 9398-095-01897593-2012 requirements and have a medical product registration certificate (RC) issued on 18.10.2019 under the number FSR 2010/09502.

The testing was performed in two steps: amplification of regions of viral DNA and fluorescent detection performed during PCR. The obtained DNA samples were used for amplification of the fragment of the viral nucleic acid with the help of region-specific primers and a *Taq*-polymerase enzyme. The reaction mix included fluorescent-labeled oligonucleotide probes, which were hybridized with a complementary region of the amplified target DNA, thus leading to increased intensity of fluorescence. To have DNA extracted from the cell-containing clinical material, the human genome region (a DNA fragment of β-globin gene (ICS Glob) – endogenous internal control sample) was amplified, thus making it possible to monitor the PCR stages (DNA extraction and PCR process) and to assess the adequacy of material collection and storage. The obtained data (fluorescent signal accumulation curves) were analyzed by using the software of the Rotor-Gene Q cycler (Qiagen, Germany). The result of amplification for a channel was considered positive, if the fluorescence curve was S-shaped as it was typical of real-time PCR and crossed one time the threshold line within the region of significantly increased fluorescence. Based on the known values of DNA concentration in standard samples (calibrators), the cycler software automatically calculated viral DNA concentrations in the studied samples per volume unit and/or per the specified number of cells.

Following the manufacturer's guidelines for the reagent kits, the DNA concentration per 1 ml of saliva sample (CONCDNA) was calculated by the formula:

$$CONCDNA = CDNA \times 100 \text{ (copies/ml)}, \quad (1)$$

where CDNA is the number of viral nucleic acid copies in a DNA sample.

Saliva samples were collected from the patients in accordance with the Collection, Transportation, and Storage of Clinical Material for PCR Diagnostics Guidelines prepared at the Central Research Institute of Epidemiology of Rospotrebnadzor. Before biomaterials were collected, the patients had rinsed their mouths three times with saline solution. The saliva in the amount of min 1.0 ml was collected in disposable, tightly sealed sterile plastic tubes.

The statistical analysis of the data was performed by using standard Excel (Microsoft 2010) and STATISTICA 10 (StatSoft 2010) programs. The sampling size was estimated with reference to the statistical power sufficient for obtaining significant differences between groups. The mean values of quantitative variables were shown as M ± 95% confidence interval. The distribution was checked for normality by using the Shapiro–Wilk test. When the distribution was significantly different from the normal pattern, we performed log transformation. The significance of differences in quantitative variables between the groups was assessed by using Student's t-test; the correlations were evaluated by using Spearman's rank correlation coefficient (rs). The differences were considered statistically significant at $p < 0.05$.

Results

The herpesvirus detection frequency in patients with Alzheimer-type cognitive disorders

The quantification of the herpesvirus genetic material in the patients' saliva showed that the EBV DNA detection frequency and its levels in aMCI and AD patients with dementia significantly exceeded the comparable variables in the control group (**Fig. 1**, **2**). The mean level of EBV DNA in Alzheimer-type cognitive disorders was by many times higher than the similar level in the control group. At the same time, there were no significant differences in the frequency of detection of nucleic acids of other herpesviruses (HHV-6, HHV-7, and CMV) in saliva; no

differences were found for the mean value of viral DNA levels in the saliva (**Fig. 2**).

The relationship between the EBV-infection with a high viral load and immunological characteristics in amnestic mild cognitive impairment

During our previous studies, we found the immunological heterogeneity of the aMCI syndrome and we described its immunological variants (**Table**). We identified unfavorable prognostic significance of the elevated levels of systemic inflammation mediators, which were accompanied by a decrease in total IgG [17]. These data and increased frequency of EBV detection in aMCI were used to analyze the relationship between EBV infection with a high viral load and immune disorders in the patients. The viral DNA level >10,000 copies/ml in saliva and/or >500 copies/10^6 cells in blood was used as a criterion (taking into account the previous data) [18].

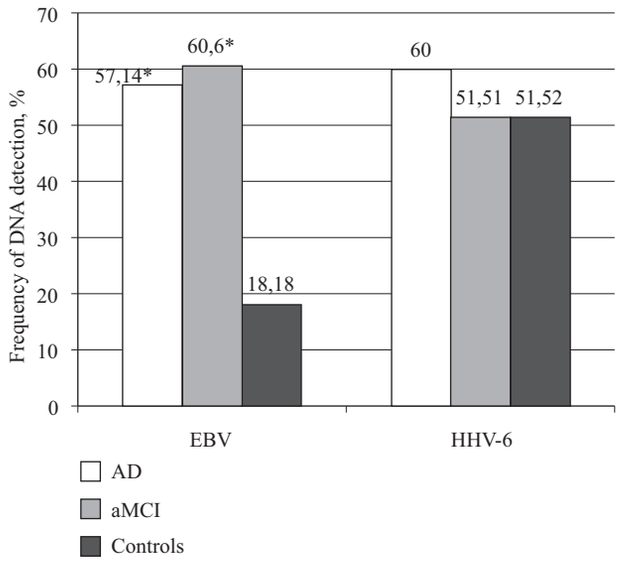

Fig. 1. The frequency of detection of Epstein–Barr virus and of human herpesvirus type 6 in the saliva of the patients with Alzheimer's disease (n = 45), amnestic mild cognitive impairment (aMCI) (n = 100) and in the control group (n = 40).

Note. * – significant differences with the control group.

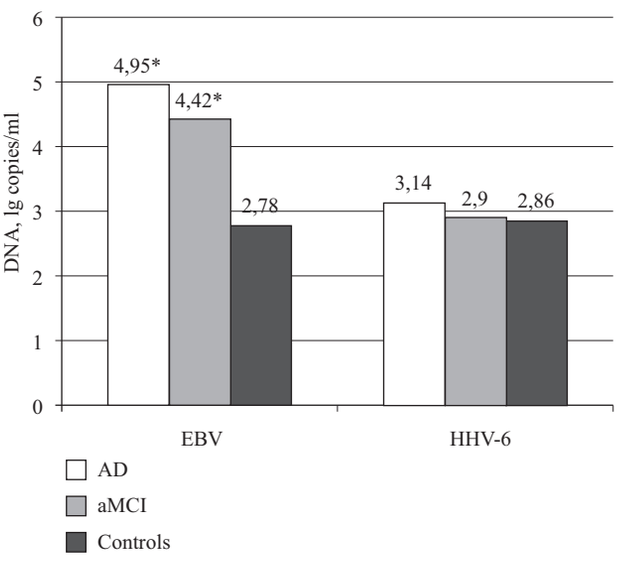

Fig. 2. The mean concentration of the DNA of Epstein–Barr virus and of human herpesvirus type 6 in the saliva of the patients with Alzheimer's disease, amnestic mild cognitive impairment (aMCI) and of the volunteers of the control group that had the DNA of these viruses in the saliva.

Note. * – significant differences with the control group.

The immunity parameters depending on the presence of systemic inflammation and on IgG levels in the patients with aMCI

Parameter	Systemic inflammation		No systemic inflammation		Controls
	IgG >10 g/l	IgG <10 g/l	IgG >10 g/l	IgG <10 g/l	
IgG, g/l	14,2 ± 0,9	9,0 ± 0,3*	13,1 ± 0,9	9,1 ± 0,5*	12,1 ± 1,4
CIC, units	116,8 ± 17,7*	115,2 ± 24,9	87,3 ± 16,3	67,6 ± 11,6	79,3 ± 16,5
CD45+CD3+CD4+, %	49,7 ± 2,3*	42,6 ± 2,5	49,2 ± 5,5	35,3 ± 5,9	42,3 ± 2,6
CD45+CD3+CD8+, %	24,2 ± 2,5	22,6 ± 2,8	24,9 ± 4,5	27,4 ± 3,5	26,5 ± 2,4
CD3+CD4+CD25+, %	2,3 ± 0,3	1,8 ± 0,3	2,5 ± 0,1*	1,4 ± 0,3	1,8 ± 0,3
CD3–CD16+CD56+, %	15,1 ± 2,8	13,9 ± 3,7	14,7 ± 7,8	16,4 ± 5,6	13,6 ± 1,6
CD3+CD16+CD56+ TNK, %	10,7 ± 2,8*	11,0 ± 3,3*	5,8 ± 1,6	7,0 ± 2,3	4,8 ± 1,0
HLA-DR+CD3–, %	13,4 ± 1,7	11,2 ± 1,3	9,7 ± 0,9*	17,3 ± 1,4*	12,2 ± 1,3
HLA-DR+CD3+, %	3,42 ± 0,7	5,5 ± 1,6	2,7 ± 0,4	4,8 ± 2,1	2,8 ± 0,6
CD19+, %	10,21 ± 1,7	7,9 ± 0,9	8,2 ± 1,0	11,5 ± 1,5*	7,9 ± 0,8
IL-10, pg/ml	6,3 ± 2,1	19,4 ± 6,7*	6,6 ± 3,2	21,1 ± 16,3	5,8 ± 0,7
IL-1β, pg/ml	13,8 ± 4,9*	14,0 ± 2,7*	5,2 ± 4,5*	17,6 ± 7,7*	3,4 ± 1,2
TNF-α, pg/ml	2,2 ± 0,5*	3,1 ± 1,0*	3,4 ± 2,0	2,1 ± 1,3	1,3 ± 0,3
C-reactive protein, mg/l	15,2 ± 5,5*	9,8 ± 3,4*	1,6 ± 0,5	1,6 ± 0,4	1,7 ± 0,1

Note. * – significant (p <0.05) differences with the control group.

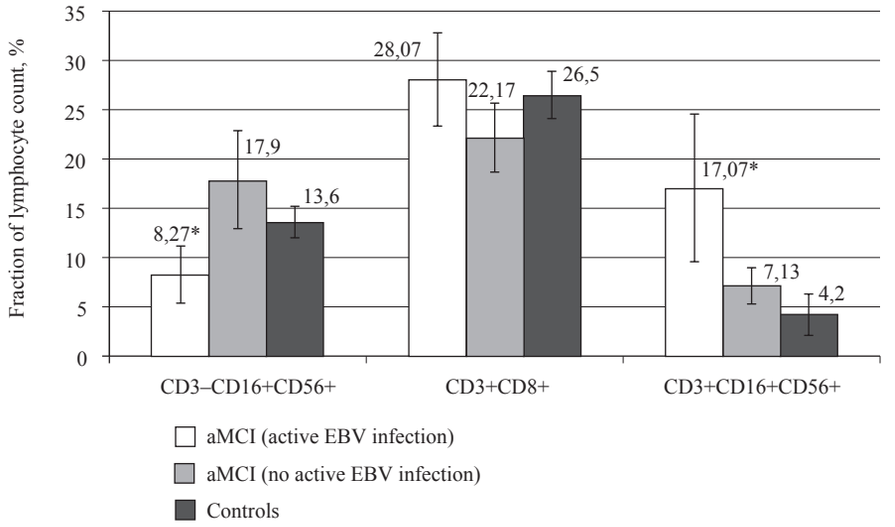

Fig. 3. The levels of CD3–CD16+CD56+ NK-cells, CD3+CD16+CD56+ TNK-cells and CD3+CD8+ cytotoxic T-cells depending on the presence of the EBV infection with high viral load in the patients with aMCI that had systemic inflammation and IgG levels >10.0 g/l.

Note. * – significant differences with the control group.

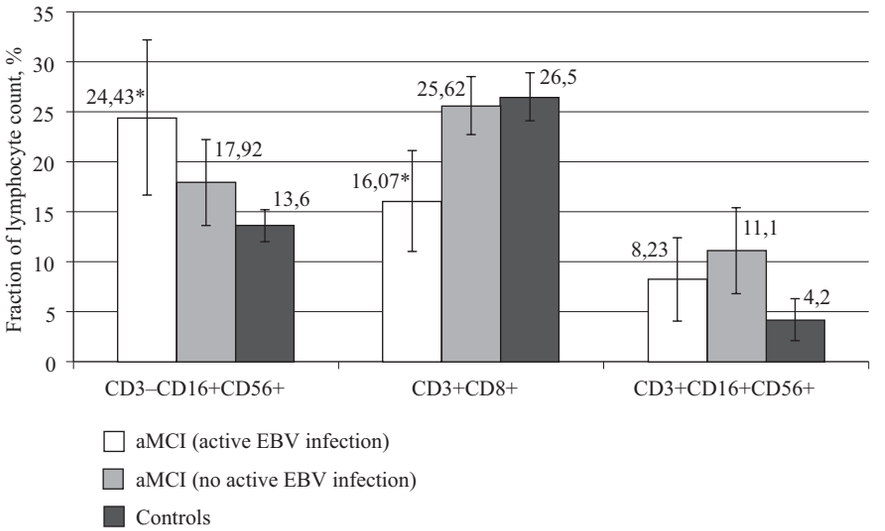

Fig. 4. The levels of CD3–CD16+CD56+ NK-cells, CD3+CD16+CD56+ TNK-cells and CD3+CD8+ cytotoxic T-cells depending on the presence of the EBV infection with high viral load in the patients with aMCI that had systemic inflammation and IgG levels <10.0 g/l.

Note. * – significant differences with the control group.

The results showed that the relationship between EBV infection and the immunity parameters was different depending on the immunological variant of the syndrome. For example, in aMCI patients with systemic inflammation (the increased levels of C-reactive protein >5 mg/l along with the increased concentration (≥2) of pro-inflammatory cytokines IL-1β, IL-8, TNF-α) and level of total IgG >10 g/l (29 patients out of 100), active EBV infection (8 patients out of 29) was associated with the decreased numbers of CD3–CD16+CD56+ NK-cells (8.27 ± 1.96%, in the control group – 17.9 ± 5.62%; p <0.05). At the same time, these patients demonstrated the tendency to elevated levels of CD3+CD8+ T-cells (cytotoxic T-lymphocytes) that increased to 28.07 ± 2.24% (in the absence of active infection caused by the above virus – 22.17 ± 1.7%; the control group – 26.5 ± 2.4%); the number of CD3+CD16+CD56+ TNK-cells also increased as compared to the control group (p <0.05) (**Fig. 3**). The identified changes could be associated with the response to the viral infection; the patients also demonstrated prevailing activation of acquired mechanisms of antiviral response together with certain signs of decreasing activation of innate pathways. These manifestations of possible imbalance in the antiviral response require further study.

In the subgroup of aMCI patients having symptoms of systemic inflammatory response and IgG <10 g/l (37 participants out of 100), active EBV infection (14 patients out of 37) was associated with decreased levels

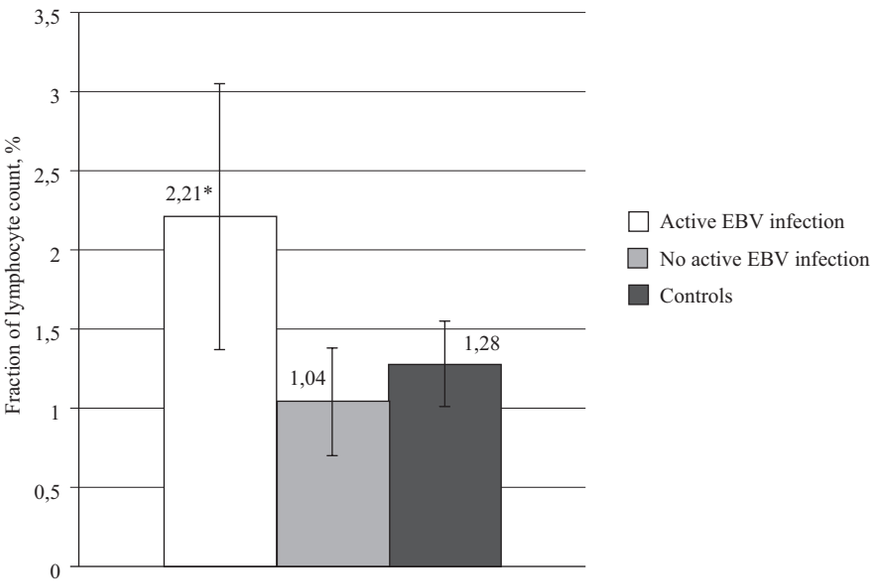

Fig. 5. The levels of tumor necrosis factor α (TNF-α) in the patients with aMCI without the signs of systemic inflammation depending on the presence of the EBV infection with high viral load.

Note. * – significant differences between the groups.

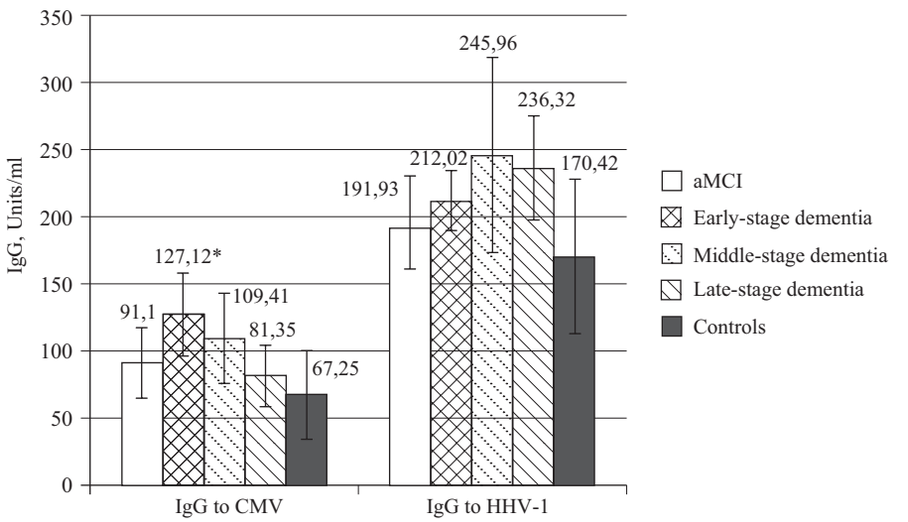

Fig. 6. The levels of immunoglobulin G to cytomegalovirus (CMV) and to herpes simplex virus type 1 (HHV-1) in the patients with aMCI and with Alzheimer's disease of different stages of dementia.

Note. * – significant differences with the control group.

of CD3+CD8+ cytotoxic T-cells to 16.07 ± 2.47% (the normal level is 26.5 ± 2.4%; p <0.05) along with elevated levels of NK-cells and normal ones of TNK-cells (**Fig. 4**). Thus, this category was characterized by prevailing innate mechanisms of antiviral immune response without any obvious signs of activation of the adaptive pathways of antiviral immunity.

Therefore, among the aMCI patients (66 out of 100) with symptoms of systemic inflammation, EBV infection with a high viral load was accompanied by imbalance in the activation of cell-mediated immune responses. It may be assumed that infection caused by this virus can be one of the factors maintaining prognostically unfavorable immune disorders in patients.

In the aMCI patients without obvious symptoms of systemic inflammation (34 patients out of 100), active EBV infection (15 patients out of 34) was associated with elevated levels of one of the most important pro-inflammatory cytokines, TNF-α; its levels were higher than the reference ones, though no relationship between the concentration levels of other studied cytokines and the immunity parameters was found (**Fig. 5**). The absence of symptoms of systemic inflammatory response in this subgroup, regardless of the level of the herpesviral load

in saliva, requires further exploration of possible genetic factors, chronic bacterial infections and other reasons that may have an impact on the extent of systemic inflammatory responses.

By and large, considering immunosuppressive properties of EBV and its ability to activate the NF-kB transcription factor by inducing the systemic inflammatory response, the results suggest the possible relationship between EBV infection and development of immune disorders in aMCI patients.

Serological markers of viral infections at different stages of Alzheimer-type cognitive disorders

The assessment of the levels of serological markers of viral infections among aMCI and AD patients showed that the CMV IgG level was significantly higher in the AD patients having mild dementia than among the patients of the control group. Its moderate stage showed only the tendency towards the CMV IgG increasing levels, while the increase was not significant. Cases of severe dementia did not demonstrate significant changes in the CMV IgG levels (**Fig. 6**). Taking into account the literature data on adverse impacts of CMV on ageing processes and neurodegeneration, the obtained results can imply a special role of CMV infection during the transition from mild cognitive impairment to dementia.

At the same time, no significant changes in the HSV IgG levels were found in the aMCI and AD patients, though these levels demonstrated an upward trend in the AD patients during all stages of dementia.

Thus, our study demonstrated the increased frequency of EBV detection in saliva and elevated levels of the viral DNA in saliva in the patients with Alzheimer-type cognitive disorders compared to the control group. It also demonstrated the relationship between active EBV infection and changes in the immunological characteristics among the aMCI patients. In addition, it was found that the CMV IgG level was associated with stages of Alzheimer-type cognitive disorders.

Discussion

The obtained results imply that EBV and CMV-caused HVI can be involved in development of immunological changes in mild cognitive impairment and progression of Alzheimer-type cognitive disorders.

With reference to the significance of identified differences in the EBV DNA detection frequency in AD patients compared to the same value in the control group, it should be noted that although adult individuals demonstrate almost 100% EBV infection prevalence, the amount of the viral nucleic acid extracted from saliva can be insufficient for detection by using an AmpliSens EBV/CMV/HHV6-screen-FL kit with analytical sensitivity of 400 DNA copies/ml. Thus, the differences in the frequency of detection of the viral DNA in saliva could result from its levels not reaching the minimum detection threshold in some of the participants.

The viral involvement in development of Alzheimer-type cognitive disorders has been studied for quite a long time, though most of the available data refer to the human herpesvirus 1 (HHV-1). The proofs of the relationship between the HHV-1 infection and AD have been studied for nearly 30 years. It was found that in herpesviral encephalitis caused by HHV-1 and in AD, the same areas of the brain were affected [19]. Later, it was demonstrated that during long-time infections the virus was able to cross the blood-brain barrier (BBB) and was detected at autopsy study of the brain of individuals aged >45 years with the probability of more than 50% [20]. This infectious agent is detected mainly in temporal and frontal lobes (the most affected during AD) [21]; these are areas that are also involved in the process in acute herpesviral encephalitis. Besides, HHV-1 ABs are detected in cerebrospinal fluid of most of the elderly people, both healthy and having AD. In brain tissues of younger people and children, the virus and its ABs are generally not detected. The ability of HHV-1 to cross BBB may depend on the efficiency of the immune response of the individual [20]. *In situ* PCR and ELISA with β-amyloid staining demonstrated that 90% of amyloid plaques in AD patients and 80% in healthy people contain HHV-1 DNA. In the meantime, 72% of the total viral nucleic acid detected in the brain is localized in plaques in AD patients compared to 24% of the amount in healthy individuals of the same age [22]. These data suggest possible participation of HHV-1 in formation of amyloid plaques and causing neuronal damage during this disease.

The tests show the relationship between the activity of HHV-1 infection and the risk of AD development. Patients with aMCI demonstrated higher avidity of IgG ABs to this virus as compared to healthy participants and AD patients. The presence of this phenomenon in the infected is seen as a marker of HHV-1 infection reactivation. Therefore, this mechanism can act as a pathogenetic factor during early stages of the disease development [23]. The data of other researchers, who conducted a 12-year-long study involving 512 senior volunteers who had normal cognitive functions as of the starting date, showed that the AD patients (77 people) demonstrated more frequently seropositivity for HHV-1 IgM antibodies (odds ratio, OR = 2.55), which may be indicative of the pathogenetic role both of the primary HHV-1 infection and of its possibly reactivation [7].

The number of studies focusing on other herpesviruses as risk factors for AD is much smaller than the number of HHV-1 studies. However, there are some data showing the role of EBV, CMV, and HHV-6 as risk factors. It was found that the HHV-6 DNA is detected in the brain of AD patients significantly more frequently than on healthy individuals. In more than fifty percent of cases, both HHV-6 and HHV-1 are detected in brain tissues of patients. However, as of today, there are no data proving the relationship between the presence of an *ApoEε4* allele (the most significant genetic risk factor for sporadic AD) and the frequency of detection of HHV-6 genetic material in the brain of patients. In one of the studies, the ABs to the above virus were detected in the cerebrospinal fluid of 22% of AD patients; however, they were not detected in the control group [20]. The results showing that AD patients have high probability of detection

EBV and HHV-6 DNAs in nuclei of white blood cells are of particular interest [6]. Furthermore, the cohort study, which lasted 5 years and included 164 senior volunteers with normal cognitive functions as of the starting date, demonstrated that at the onset of the study, the probability of detection of EBV and/or HHV-6 nucleic acids in nuclei of white blood cells was much higher among the patients who developed AD during 5 years. Seropositivity for EBV or HHV-6-specific IgGs was associated with increased risk of the disease [6].

There is indirect evidence of potential CMV significance in AD pathogenesis. For example, elevated serum levels of neopterin, a catabolic product of guanosine triphosphate synthesized by macrophages as an antimicrobial agent, are nonspecific markers of active viral infection. Some studies have demonstrated that neopterin can serve as an indicator of acute CMV infection. In AD patients, its levels correlate with CMV IgM AB levels and the degree of cognitive impairment, thus implying the role of the latter in AD progression [8]. There are also data showing that AD is characterized by the association between CMV IgG and HHV-1 IgG AB detection. Note that among the CMV-positive individuals with the above disease, the probability of anti-HHV-1 IgG detection is higher than among the individuals from the control group [24]. These data suggest that the CMV and HHV-1 herpesvirus association can play a role in the AD pathogenesis. It should be pointed out that the discussed study [24] did not find any differences between the study groups and the control ones in levels of CMV antibodies, thus contravening our results. The controversy can be explained by the fact that the researchers did not divide the participants into groups depending on their stage of dementia [24]. Our data show that although patients with initial symptoms of dementia had elevated CMV IgG levels, patients with severe dementia demonstrated a downward trend in CMV IgG levels, which could be associated with a lower level of total IgG production in this category of participants [3]. The absence of differences in HHV-1 IgG levels during different stages of cognitive disorders is consistent with findings of other researchers, which demonstrate that the AD development is accompanied by increasing HHV-1 IgM levels. This fact proves the role of acute HHV1 infection or its recurrences in the disease pathogenesis [7].

Conclusion

The analysis of literature data confirms that there is evidence of the relationship between HHV-1 infection of the central nervous system (CNS) and AD progression, including the adverse effect of active HHV-infection on the probability of its development. The relationship of EBV, CMV, HHV-6, and HHV-7 with the risk of this disease is significantly understudied. Furthermore, the influence of HVI on systemic immunity characteristics in Alzheimer-type cognitive disorders has been poorly studied, though there are data on immunotropic effects of herpesviruses and on aMCI and AD-associated changes in immunological parameters.

For the first time, we obtained the data proving the increased frequency of EBV detection in saliva, higher levels of viral DNA in the saliva of aMCI and AD patients as well as the relationship of EBV infection with the extent and nature of immune disorders in different (including those associated with high risk of cognitive disorder progression) immunologic versions of the aMCI syndrome. Higher CMV IgG levels were detected in AD patients having mild dementia. The results suggest that HVI caused by EBV and CMV may play an important role in development of immunological changes in mild cognitive impairment and progression of Alzheimer-type cognitive disorders. The obtained data can be useful for prognostic methods addressing AD development, including its pre-dementia stage, and for new approaches to individualized treatment and prevention.

REFERENCES

1. Manly J.J, Tang M., Schupf N., Stern Y., Vonsattel J.G., Mayeux R. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann. Neurol.* 2008; 63(4): 494–506. https://doi.org/10.1002/ana.21326.
2. Alzheimer's Disease International. *World Alzheimer Report 2018. The State of the Art of Dementia Research: New Frontiers*. London: Alzheimer's Disease International (ADI); 2018. Available at: https://www.alz.co.uk/research/WorldAlzheimerReport2018.pdf (accessed March 22, 2021).
3. Malashenkova I.K., Krynskiy S.A., Khaylov N.A., Ogurtsov D.P., Ponomareva E.V., Gavrilova S.I., et al. Adaptive immunity, systemic inflammation and cytokine levels in patients with Alzheimer's disease of different severity and with amnestic mild cognitive impairment *[Adaptivnyy immunitet, sistemnoe vospalenie i uroven' osnovnykh tsitokinov u patsientov s bolezn'yu Altsgeimera razlichnykh stadiy i myagkim kognitivnym snizheniem amnesticheskogo tipa]*. *Allergologiya i immunologiya.* 2018; 19(4): 206–14 (in Russian).
4. Malashenkova I.K., Krynskiy S.A., Hailov N.A., Ogurtsov D.P., Selezneva N.D., Fedorova Y.B., et al. Anti-inflammatory effects of neurotrophic therapy (a pilot study) *[Protivovospalitel'nye effekty neirotroficheskoy terapii (primenenie tserebrolizina pri myagkom kognitivnom snizhenii)]*. *Zhurnal nevrologii i psikhiatrii im. S.S. Korsakova.* 2018; 118(5): 39–44. https://doi.org/10.17116/jnevro20181185139 (in Russian).
5. Simonov A.N., Klyushnik T.P., Androsova L.V., Mikhaylova N.M. The use of cluster analysis and logistic regression for assessing the risk of Alzheimer's disease in patients with mild cognitive impairment, amnestic type *[Ispol'zovanie klasternogo analiza i logisticheskoy regressii dlya otsenki riska bolezni Altsgeimera u patsientov s sindromom myagkogo kognitivnogo snizheniya amnesticheskogo tipa]*. *Zhurnal nevrologii i psikhiatrii im. S.S. Korsakova.* 2018; 118(12): 40–3. https://doi.org/10.17116/jnevro201811812140 (in Russian).
6. Carbone I., Lazzarotto T., Ianni M., Porcellini E., Forti P., Masliah E., et al. Herpes virus in Alzheimer's disease: relation to progression of the disease. *Neurobiol. Aging*. 2014; 35(1): 122–9. https://doi.org/10.1016/j.neurobiolaging.2013.06.024.
7. Letenneur L., Pérès K., Fleury H., Garrigue I., Barberger-Gateau P., Helmer C., et al. Seropositivity to herpes simplex virus antibodies and risk of Alzheimer's disease: a population-based cohort study. *PLoS One*. 2008; 3(11): e3637. https://doi.org/10.1371/journal.pone.0003637.
8. Blasko I., Knaus G., Weiss E., Kemmler G., Winkler C., Falkensammer G., et al. Cognitive deterioration in Alzheimer's disease is accompanied by increase of plasma neopterin. *J. Psychiatr. Res*. 2007; 41(8): 694–701. https://doi.org/10.1016/j.jpsychires.2006.02.001.
9. Stein P.S., Steffen M.J., Smith C., Jicha G., Ebersole J.L., Dolph D. Serum antibodies to periodontal pathogens are a risk factor for Alz-

heimer's disease. *Alzheimer's Dement*. 2012; 8(3): 196–203. https://doi.org/10.1016/j.jalz.2011.04.006.
10. Kamer A.R., Craiga R.G., Pirraglia E., Dasanayake A.P., Norman R.G., Boylan R.J., et al. TNF-α and antibodies to periodontal bacteria discriminate between Alzheimer's disease patients and normal subjects. *J. Neuroimmunol*. 2009; 216(1–2): 92–7. https://doi.org/10.1016/j.jneuroim.2009.08.013.
11. Didkovskii N.A., Malashenkova I.K., Tanasova A.N., Zuikov I.A., Zuikova I.N., Khitrik N.M., et al. Pathogenetic aspects of severe course of herpetic infection. *Bull. Exp. Biol. Med*. 2007; 2(76): 76–81. https://doi.org/10.1007/s10517-007-0349-7.
12. Hemling N., Röyttä M., Rinne J., Pöllänen P., Broberg E., Tapio V., et al. Herpesviruses in brains in Alzheimer's and Parkinson's diseases. *Ann. Neurol.* 2003; 54(2): 267–71. https://doi.org/10.1002/ana.10662.
13. Lin W.R., Wozniak M.A., Cooper R.J., Wilcock G.K., Itzhaki R.F. Herpesviruses in brain and Alzheimer's disease. *J. Pathol.* 2002; 197(3): 395–402. https://doi.org/10.1002/path.1127.
14. Zilka N., Kazmerova Z., Jadhav S., Neradil P., Madari A., Obetkova D., et al. Who fans the flames of Alzheimer's disease brains? Misfolded tau on the crossroad of neurodegenerative and inflammatory pathways. *J. Neuroinflammation.* 2012; 9: 47. https://doi.org/10.1186/1742-2094-9-47.
15. Kuhla A., Ludwig S.C., Kuhla B., Münch G., Vollmar B. Advanced glycation end products are mitogenic signals and trigger cell cycle reentry of neurons in Alzheimer's disease brain. *Neurobiol. Aging.* 2015; 36(2): 753–61. https://doi.org/10.1016/j.neurobiolaging.2014.09.025.
16. Doens D., Fernández P.L. Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *J. Neuroinflammation.* 2014; 11: 48. https://doi.org/10.1186/1742-2094-11-48.
17. Krynskiy S.A., Malashenkova I.K., Khaylov N.A., Ogurtsov D.P., Chekulaeva E.I., Ponomareva E.V., et al. Immunological markers of long-term effects of treatment in patients with mild cognitive impairment *[Immunologicheskie markery dolgosrochnykh effektov terapii u patsientov s sindromom myagkogo kognitivnogo snizheniya]*. *Meditsinskiy akademicheskiy zhurnal.* 2019; 19(S1): 84–6 (in Russian).
18. Didkovsky N.A., Ogurtsov D.P., Krynskiy S.A., Gurskaya O.G., Shipulina O.Yu., Domonova E.A., et al. Myalgic encephalomyelitis/chronic fatigue syndrome: replication levels of lymphotropic herpesviruses and immune defense *[Mialgicheskiy entsefalomielit/sindrom khronicheskoy ustalosti: uroven' replikatsii limfotropnykh gerpeticheskikh virusov i immunnaya zashchita]*. *Poliklinika.* 2016; (5): 46–50 (in Russian).
19. Denaro F.J., Staub P., Colmer J., Freed D.M. Coexistence of Alzheimer disease neuropathology with herpes simplex encephalitis. *Cell. Mol. Biol. (Noisy-le-Grand)*. 2003; 49(8): 1233–40.
20. Wozniak M.A., Shipley S.J., Combrinck M., Wilcock G.K., Itzhaki R.F. Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients. *J. Med. Virol.* 2005; 75(2): 300–6. https://doi.org/10.1002/jmv.20271.
21. Jamieson G.A., Maitland N.J., Wilcock G.K., Yates C.M., Itzhaki R.F. Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type. *J. Pathol.* 1992; 167(4): 365–8. https://doi.org/10.1002/path.1711670403.
22. Honjo K., van Reekum R., Verhoeff N. Alzheimer's disease and infection: Do infectious agents contribute to progression of Alzheimer's disease? *Alzheimer's Dement.* 2009; 5(4): 348–60. https://doi.org/10.1016/j.jalz.2008.12.001.
23. Kobayashi N., Nagata T., Shinagawa S., Oka N., Shimada K., Shimizu A., et al. Increase in the IgG avidity index due to herpes simplex virus type 1 reactivation and its relationship with cognitive function in amnestic mild cognitive impairment and Alzheimer's disease. *Biochem. Biophys. Res. Commun*. 2013; 430(3): 907–11. https://doi.org/10.1016/j.bbrc.2012.12.054.
24. Lövheim H., Olsson J., Weidung B., Johansson A., Eriksson S., Hallmans G., et al. Interaction between cytomegalovirus and herpes simplex virus type 1 associated with the risk of Alzheimer's disease development. *J. Alzheimers Dis*. 2018; 61(3): 939–45. https://doi.org/10.3233/JAD-161305.

ЛИТЕРАТУРА

1. Manly J.J, Tang M., Schupf N., Stern Y., Vonsattel J.G., Mayeux R. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann. Neurol.* 2008; 63(4): 494–506. https://doi.org/10.1002/ana.21326.
2. Alzheimer's Disease International. *World Alzheimer Report 2018. The State of the Art of Dementia Research: New Frontiers*. London: Alzheimer's Disease International (ADI); 2018. Available at: https://www.alz.co.uk/research/WorldAlzheimerReport2018.pdf (accessed March 22, 2021).
3. Малашенкова И.К., Крынский С.А., Хайлов Н.А., Огурцов Д.П., Пономарева Е.В., Гаврилова С.И., и др. Адаптивный иммунитет, системное воспаление и уровень основных цитокинов у пациентов с болезнью Альцгеймера различных стадий и мягким когнитивным снижением амнестического типа. *Аллергология и иммунология.* 2018; 19(4): 206–14.
4. Малашенкова И.К., Крынский С.А., Хайлов Н.А., Огурцов Д.П., Селезнева Н.Д., Фёдорова Я.Б., и др. Противовоспалительные эффекты нейротрофической терапии (применение церебролизина при мягком когнитивном снижении). *Журнал неврологии и психиатрии им. С.С. Корсакова.* 2018; 118(5): 39–44. https://doi.org/10.17116/jnevro20181185139.
5. Симонов А.Н., Клюшник Т.П., Андросова Л.В., Михайлова Н.М. Использование кластерного анализа и логистической регрессии для оценки риска болезни Альцгеймера у пациентов с синдромом мягкого когнитивного снижения амнестического типа. *Журнал неврологии и психиатрии им. С.С. Корсакова.* 2018; 118(12): 40–3. https://doi.org/10.17116/jnevro201811812140.
6. Carbone I., Lazzarotto T., Ianni M., Porcellini E., Forti P., Masliah E., et al. Herpes virus in Alzheimer's disease: relation to progression of the disease. *Neurobiol. Aging.* 2014; 35(1): 122–9. https://doi.org/10.1016/j.neurobiolaging.2013.06.024.
7. Letenneur L., Pérès K., Fleury H., Garrigue I., Barberger-Gateau P., Helmer C., et al. Seropositivity to herpes simplex virus antibodies and risk of Alzheimer's disease: a population-based cohort study. *PLoS One.* 2008; 3(11): e3637. https://doi.org/10.1371/journal.pone.0003637.
8. Blasko I., Knaus G., Weiss E., Kemmler G., Winkler C., Falkensammer G., et al. Cognitive deterioration in Alzheimer's disease is accompanied by increase of plasma neopterin. *J. Psychiatr. Res.* 2007; 41(8): 694–701. https://doi.org/10.1016/j.jpsychires.2006.02.001.
9. Stein P.S., Steffen M.J., Smith C., Jicha G., Ebersole J.L., Dolph D. Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. *Alzheimer's Dement.* 2012; 8(3): 196–203. https://doi.org/10.1016/j.jalz.2011.04.006.
10. Kamer A.R., Craiga R.G., Pirraglia E., Dasanayake A.P., Norman R.G., Boylan R.J., et al. TNF-α and antibodies to periodontal bacteria discriminate between Alzheimer's disease patients and normal subjects. *J. Neuroimmunol.* 2009; 216(1–2): 92–7. https://doi.org/10.1016/j.jneuroim.2009.08.013.
11. Didkovskii N.A., Malashenkova I.K., Tanasova A.N., Zuikov I.A., Zuikova I.N., Khitrik N.M., et al. Pathogenetic aspects of severe course of herpetic infection. *Bull. Exp. Biol. Med.* 2007; 2(76): 76–81. https://doi.org/10.1007/s10517-007-0349-7.
12. Hemling N., Röyttä M., Rinne J., Pöllänen P., Broberg E., Tapio V., et al. Herpesviruses in brains in Alzheimer's and Parkinson's diseases. *Ann. Neurol.* 2003; 54(2): 267–71. https://doi.org/10.1002/ana.10662.
13. Lin W.R., Wozniak M.A., Cooper R.J., Wilcock G.K., Itzhaki R.F. Herpesviruses in brain and Alzheimer's disease. *J. Pathol.* 2002; 197(3): 395–402. https://doi.org/10.1002/path.1127.
14. Zilka N., Kazmerova Z., Jadhav S., Neradil P., Madari A., Obetkova D., et al. Who fans the flames of Alzheimer's disease brains?

Misfolded tau on the crossroad of neurodegenerative and inflammatory pathways. *J. Neuroinflammation.* 2012; 9: 47. https://doi.org/10.1186/1742-2094-9-47.

15. Kuhla A., Ludwig S.C., Kuhla B., Münch G., Vollmar B. Advanced glycation end products are mitogenic signals and trigger cell cycle reentry of neurons in Alzheimer's disease brain. *Neurobiol. Aging.* 2015; 36(2): 753–61. https://doi.org/10.1016/j.neurobiolaging.2014.09.025.
16. Doens D., Fernández P.L. Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *J. Neuroinflammation.* 2014; 11: 48. https://doi.org/10.1186/1742-2094-11-48.
17. Крынский С.А., Малашенкова И.К., Хайлов Н.А., Огурцов Д.П., Чекулаева Е.И., Пономарева Е.В., и др. Иммунологические маркеры долгосрочных эффектов терапии у пациентов с синдромом мягкого когнитивного снижения. *Медицинский академический журнал*. 2019; 19(S1): 84–6.
18. Дидковский Н.А., Огурцов Д.П., Крынский С.А., Гурская О.Г., Шипулина О.Ю., Домонова Э.А., и др. Миалгический энцефаломиелит/синдром хронической усталости: уровень репликации лимфотропных герпетических вирусов и иммунная защита. *Поликлиника.* 2016; (5): 46–50.
19. Denaro F.J., Staub P., Colmer J., Freed D.M. Coexistence of Alzheimer disease neuropathology with herpes simplex encephalitis. *Cell. Mol. Biol. (Noisy-le-Grand).* 2003; 49(8): 1233–40.
20. Wozniak M.A., Shipley S.J., Combrinck M., Wilcock G.K., Itzhaki R.F. Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients. *J. Med. Virol.* 2005; 75(2): 300–6. https://doi.org/10.1002/jmv.20271.
21. Jamieson G.A., Maitland N.J., Wilcock G.K., Yates C.M., Itzhaki R.F. Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type. *J. Pathol.* 1992; 167(4): 365–8. https://doi.org/10.1002/path.1711670403.
22. Honjo K., van Reekum R., Verhoeff N. Alzheimer's disease and infection: Do infectious agents contribute to progression of Alzheimer's disease? *Alzheimer's Dement.* 2009; 5(4): 348–60. https://doi.org/10.1016/j.jalz.2008.12.001.
23. Kobayashi N., Nagata T., Shinagawa S., Oka N., Shimada K., Shimizu A., et al. Increase in the IgG avidity index due to herpes simplex virus type 1 reactivation and its relationship with cognitive function in amnestic mild cognitive impairment and Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 2013; 430(3): 907–11. https://doi.org/10.1016/j.bbrc.2012.12.054.
24. Lövheim H., Olsson J., Weidung B., Johansson A., Eriksson S., Hallmans G., et al. Interaction between cytomegalovirus and herpes simplex virus type 1 associated with the risk of Alzheimer's disease development. *J. Alzheimers Dis*. 2018; 61(3): 939–45. https://doi.org/10.3233/JAD-161305.