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Research Article

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Interleukin-1 Beta (IL-1β) and Cognitive Complaints in Patients with Chronic Human Herpesviruses 6 Infection

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ABSTRACT

Background and Aims: Cytokines are signaling molecules produced by immune and non-immune cells that affect the periphery and brain, participating in the regulation of acute and chronic inflammation. This study examined IL-1β production in patients with reactivated chronic Human Herpesviruses 6 infection (HHV6).

Methods: The study included 66 patients with chronic HHV6 infection (50 women and 16 men). Average age: 34.67 ± 1.21 years. Disease duration: 2.83 ± 0.14 years. All patients were tested for HHV6 DNA copy number using polymerase chain reaction (PCR) in saliva samples and for interleukin-1β (IL-1β) production in culture medium.

Results: The HHV6 DNA copy number in patients was 1114.24±180.27 (95% CI: 767.74 - 1491.17). The serum HHV6 IgG antibody content was 4.20±0.29 (95% CI: 3.65 - 4.78) and was present in all patients, regardless of the severity of cognitive complaints. The content of serum and spontaneous IL-1β was within the reference values. The level of induced IL-1β was elevated in all patients (2382.03±277.90 pg/ml, (95% CI: 1909.35–2985.25)) and exceeded the reference values by 1.98 times. Linear and logistic regression showed a relationship between the level of induced IL-1β and the number of HHV6 DNA copies (p=0.001 and p=0.000), which was confirmed by ANOVA analysis of variance (p=0.001). An association between induced IL-1β and cognitive complaints was found and confirmed by ANOVA.

Conclusions: Logistic regression and variance analysis revealed a relationship between induced IL-1β and cognitive complaints in patients.

Abbreviations

HTLV-III	:	The human T-cell lymphotropic virus type III
HHV6	:	human herpes virus 6
CNS	:	central nervous system
DNA	:	deoxyribonucleic acid
BBB	:	blood-brain barrier
IL-1β	:	interleukin-1β
TNF-α	:	tumor necrosis factor-alpha
IL-6	:	interleukin-6
TJ	:	tight junction (cytoplasmic proteins)
CVO	:	Circumventricular organs
PAMP	:	pathogen-associated molecular pattern
MCP-1	:	monocyte chemoattractant protein-1
CCR2	:	C-C chemokine receptor type 2
CFS/ME	:	chronic fatigue syndrome/myalgic encephalomyelitis
IgG	:	immunoglobulin G
PCR	:	polymerase chain reaction method

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Keywords: Herpes Virus Type 6, Neuroinflammation, Cognitive Complaints, Interleukin-1 Beta, HHV-6 DNA Copy Number

In 1986, S.Z. Salahuddin et al. published the results of a study on a new lymphotropic virus that was isolated from the peripheral blood of six patients in the United States. Two patients were HTLV-III seropositive (AIDS-associated lymphoma and dermatopathic lymphadenopathy), three patients were HTLV-III seronegative (angioimmunoblastic lymphadenopathy, cutaneous T-cell lymphoma, immunoblastic lymphoma), and one HTLV-III seronegative patient with acute lymphoblastic leukemia from Jamaica [1]. The virus was identical to herpes viruses and was named "human herpesvirus 6" (HHV6) (strain GS) and officially classified in the order *Herpesvirales*, family *Herpesviridae,* subfamily *Betaherpesvirinae*, genus *Roseolovirus* [2]. In 1987, in Africa, Downing R.G. et al. isolated independent isolates called U1102 (from Uganda) from AIDS patients [2]. In 1988, in Zaire, Lopez C. et al. isolated Z29 isolates from peripheral blood mononuclear cells of patients with roseola, after bone marrow transplantation, with leukopenia, and from an HIV-1-positive patient with AIDS [3]. The obtained isolates proliferated in T cells (CEM, H9, Jurkat), monocytes (HL60, U937), glial cells (HED), and also in B cells (Raji, RAMOS, L4, WHPT) [2, 4]. Ablashi DV et al proposed dividing them into group A (type GS) and group B (type Z-29) [5]. In 1992, it was suggested that these two groups of HHV-6 are variants of the same species: HHV-6A and HHV-6B [6]. In 2012, the International Committee on Taxonomy of Viruses officially approved the classification of HHV-6A and HHV-6B as separate viruses [7].

The main route of infection occurs through the mucous membrane of the tonsils, olfactory cells of the nasal mucosa. That is, upon contact of infected saliva or nasal mucus with the respiratory tract.

HHV-6A/B infects immune system cells CD4+ T cells, CD8+ T cells and NK cells; cells of the nervous system - astrocytes, microglial cells, oligodendrocytes and neurons; cells of other tissues - liver cells, human fibroblasts, epithelial cells and endothelial cells, cells of the reproductive tract [8]. Over 95% of adults are infected with HHV-6B. A smaller part of the population is infected with HHV-6A. Primary infection occurs between the ages of 6 months and 2 years. At the age of up to 1 month, infection rate is 10%, and at the age of 12 months - 66%. Infection with variant B is approximately three times more common than with variant A [9].

HHV-6 infection of the central nervous system (CNS) occurs via peripheral axons and the bloodstream through two distinct routes: hematogenous dissemination, in which the virus enters the brain through the blood–brain barrier (BBB), and retrograde neuronal dissemination, in which the virus infects peripheral nerve endings via axonal transport networks to enter the brain [10].

Through olfactory receptor neurons, viruses directly penetrate the olfactory and limbic systems of the CNS, which has been confirmed by the presence of HHV-6 in the olfactory bulb [11]. A study of brain biopsy tissue from 118 patients with neural tumors revealed the presence of HHV-6 DNA in 32% of cases. In biopsy tissue from healthy individuals taken at autopsy, the presence of HHV-6 DNA was detected in 37% of cases [12]. According to the results of another study, HHV-6 DNA (both HHV-6A and HHV-6B) was detected in the brain of 85% of patients who died of natural causes. HHV-6B was detected in 75% of patients. The authors suggested that HHV-6A and HHV-6B are neurotropic [13]. The viruses cause neuronal damage through viral replication and cell lysis. When astrocytes, oligodendrocytes, or endothelial cells are infected with the virus, the production of proinflammatory cytokines, including IL-1β, IFN-α, and TNF-α is induced [14, 15].

The development of inflammation alters the properties of the BBB, promoting leukocyte infiltration and cytokine invasion, increasing its permeability. Extravasation of immune cells into the brain results in the migration of anti-inflammatory cells (neutrophils, T cells, B cells, dendritic cells, and macrophages) into the brain, which promotes BBB restoration [16, 17].

Peripheral proinflammatory cytokines can penetrate the brain through the BBB via the following possible pathways:

- the neural pathway, which involves stimulation of the afferent vagus nerve, transmitting neural signals through the lower brainstem to the hypothalamus and amygdala;
- the humoral pathway, which involves the production of proinflammatory cytokines by phagocytic cells in the circumventricular organs (CVO) and choroid plexus, followed by the dissemination of these immune signals into the brain parenchyma [18,19].

Charlotte D'Mello et al. demonstrated the existence of a third, cellular pathway in a mouse model by stimulating microglia with proinflammatory cytokines, with increased levels of monocyte chemotactic protein (MCP)-1 in the brain, as well as an increase in the number of circulating monocytes expressing C-C chemokine receptor type 2 (CCR2) in the meninges of the brain parenchyma [20].

Cytokines, entering the brain through the BBB via the circumventricular organs or synthesized directly in the brain, participate in the development of an inflammatory response. This reaction leads to an immune response in the CNS, called neuroinflammation, and forms a mirror image of the cytokine profile in the periphery. Microglia play a key role in the development of brain neuroinflammation, producing inflammatory mediators such as IL-1β, TNF, IL-6, and monocyte chemotactic protein-1 (MCP-1), which contribute to BBB damage [21]. Activation of IL-1β expression during local or peripheral injury damages microglia, astrocytes, and endothelial cells, which maintain the integrity of the blood-brain barrier. Glial cells, in response to prolonged stress and injury, exacerbate inflammatory processes, leading to the development of neuroinflammation.

Elevated IL-1β levels lead to the progression of neuroinflammation and impaired brain function [22]. Psychological and physical stressors promote increased cytokine production and prolonged activity. This can result in disruption of neuronal function, signaling, synthesis, reuptake, and release of neurotransmitters. This leads to disruption of neural connections responsible for cognitive function and mood [23]. Behavioral changes during infection result from direct interactions between proinflammatory cytokines and neurons. TNFα, IL-1β, or both are required for the

development of sickness-like and depressive behaviors [21].

Acute or chronic stress can lead to increased production of proinflammatory cytokines, which, when released into the brain, lead to changes in neurotransmitters involved in the development of depression.

This has been suggested based on the following cytokine effects:
- they cause fatigue, drowsiness, and symptoms of anxiety / depression;
- they can cause changes in the neuroendocrine system and central neurotransmitters associated with depression;
- they cause major depressive disorder [24].

IL1β, produced by glia, interacts with its receptors on neurons. This results in altered regulation of neuromodulators and neurotransmitter receptors, leading to changes in neuronal excitability and function [25].

An analysis of the scientific literature was conducted on the presence and severity of cognitive complaints in patients with reactivation of chronic HHV-6 infection and the association between IL1β cytokine levels and cognitive complaints. We found no similar research results in the literature.

The aim of this study is to investigate the development of cognitive complaints in patients with chronic HHV-6 infection and the relationship between the production of the proinflammatory cytokine IL1β and the severity of these complaints.

Material and Methods Patients.

A single-center cross-sectional study included 66 patients with chronic HHV-6 infection. Participants were selected for the study based on the following inclusion criteria:
- age 25–50 years;
- HHV6 DNA copy number in a saliva sample of at least 10^2 –10^3;
- presence of IgG antibodies to HHV6 in serum prior to examination by an immunologist;
- presence of clinical complaints associated with HHV6;
- signed informed consent prior to the study.

Exclusion criteria were age under 18 years; combined infection; pregnancy; type 2 diabetes mellitus; other autoimmune diseases; acute infectious conditions. A total of 129 patients with HHV6 infection were examined during the study period. Of these, 48 patients (37.2%) were excluded because they did not meet the inclusion criteria:
- Co-infection (hepatitis B or C, chlamydia) - 12 people;
- Combination with Lyme disease - 2;
- Acute cystitis - 3;
- Autoimmune diseases (autoimmune thyroiditis, Crohn's disease, rheumatoid arthritis) - 14 people;
- Endometriosis - 6;
- Pregnancy - 3;
- Diabetes mellitus - 8.

Moreover, 15 patients (11.6%) developed another condition (vasculitis, hypothyroidism, endometriosis, etc.) within three months of study inclusion. Thus, the final sample included 66 patients (Figure 1). There were 50 women and 16 men. Women get sick and seek medical care more often than men. The average age of patients was 34.67 ± 1.21 years (95% CI: 32.24 - 37.01).

The age of men was 35.05 ± 2.64 (95% CI: 30.00 - 40.18), the age of women was 33.615 ± 1.34 (95% CI: 30.90 -36.21) (p = 0.605). The duration of the disease in the overall group was 2.83 ± 0.14 years (95% CI: 2.55 -3.14). In men, the duration was -2.13±1.98 (95% CI:1.73-2.50), in women, the duration was 2.99±0.17 (95% CI:2.66-3.34) (p=0.02).

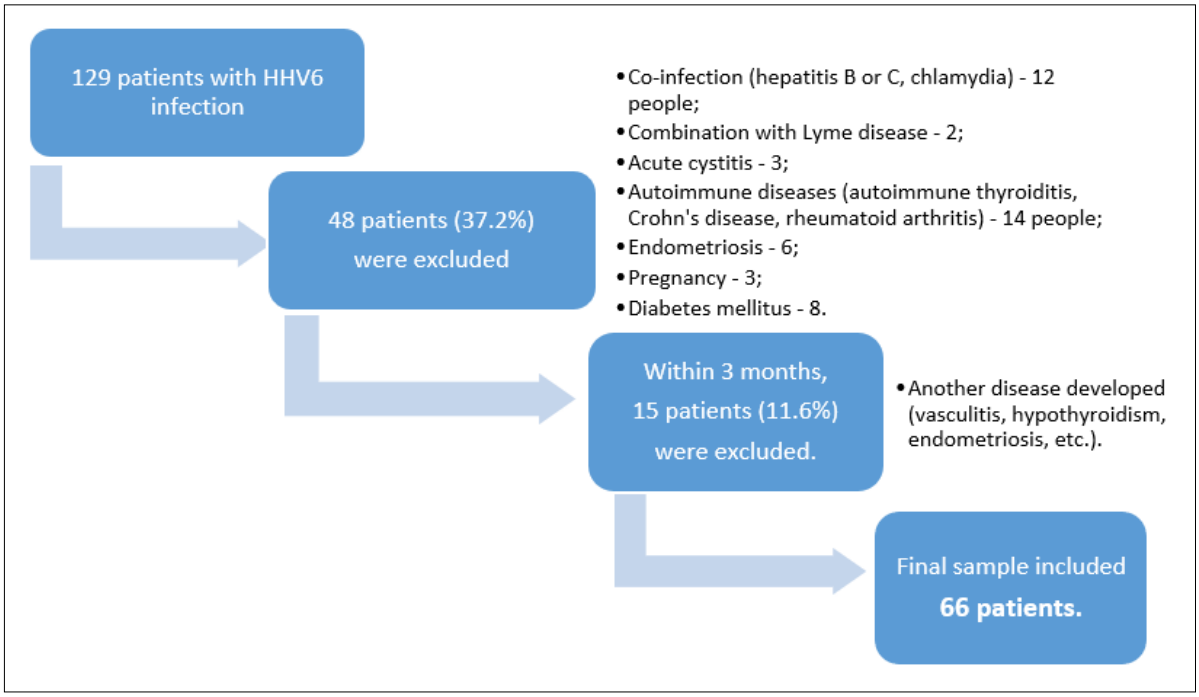

Figure 1: Selection of patients for the study

All patients were referred to an immunologist with a diagnosis of reactivation of chronic HHV6 infection. The diagnosis was confirmed by a local infectious disease expert. All patients tested positive for IgG antibodies to HHV-6 in their serum. HHV-6 DNA was detected in saliva samples from patients using the polymerase chain reaction method. No copies of HHV6 DNA were detected in the patients' blood mononuclear cells using the PCR method. This can be explained by the fact that the presence of latent HHV6 DNA in lymphocytes is a common phenomenon, but infected lymphocytes are rare (less than or equal to 1 infected cell per 10^5 lymphocytes) [26].

All patients were also examined by a neurologist due to complaints of impaired memory or concentration, sleep disorders, etc. It should be noted that during an outpatient examination by a neurologist, not a single patient was diagnosed with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Given the nature of the complaints, all patients were examined for cognitive impairment. The Mini-mental state examination (MMSE), developed in 1975, was used [27]. The MMSE scale remains one of the most widely used tools for assessing cognitive functions and is still considered the gold standard in clinical practice. A score of 24 on the scale corresponds to mild cognitive impairment. According to the examination results, the average score was 25.40±9.11 (95% CI: 25.18–25.63). In men, the average score was 25.11±0.24 (95% CI: 24.60–25.58). In women, the average score was 25.05±0.12 (95% CI: 24.79–25.31) (p=0.816). No other neurological diseases were detected.

During the initial consultation with an immunologist, patients presented with predominantly cognitive complaints. When asked, "What preceded the onset of your complaints and deterioration of your condition?" patients consistently cited "depression/chronic stress (job loss, divorce, serious illness in family members, death of a family member, etc.). Stress was present in all patients examined. All patients with chronic HHV-6 in the study were being monitored by a psychiatrist and had been taking antidepressants (selective serotonin reuptake inhibitors) for a long period of time (at least 3 months or more).

At the start of the study, patients had not received any antiviral or immunomodulatory therapy for 6 months.

The patients' clinical condition was assessed using a standard methodology, including objective data and patient complaints at the time of examination. Complaints were recorded using a subjective assessment scale on a 3-point scale: 0 – no symptoms, 1 – mild symptoms, 2 – moderate symptoms, 3 – severe symptoms.

Ethical Consideration

This cross-sectional study included 66 patients diagnosed with reactivation of chronic HHV-6 infection between March 1, 2023, and August 31, 2025, who met the inclusion criteria. The study was conducted at the Department of Allergology, Immunology, and Clinical Transfusiology, Municipal Outpatient Hospital in St. Petersburg. It was approved by the Ethics Committee of the local Ethics Committee of the St. Petersburg Dialysis Center LLC FRESENIUS MEDICAL CARE (protocol No. 9 dated 12.02.2023). All patients signed written informed consent agreeing to complete the questionnaire and allow the use of their medical data (e.g., immunological examination data) for the study.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (2013), the Protocol to the Council of Europe Convention on Human Rights and Biomedicine of 1999, Articles 20, 22, 23 of the Federal Law "On the Fundamentals of Health Protection of Citizens in the Russian Federation" dated November 21, 2011 No. 323-FZ (as amended on May 26, 2021).

Detection of Viral DNA by PCR in Saliva Samples

The study used a DNA copy number assay that does not differentiate HHV-6A from HHV-6B. HHV6 DNA has been shown to be present in saliva and salivary glands. Sada et al., using PCR, demonstrated that HHV6 DNA was present in 88.1% of submandibular gland samples, 63.0% of parotid gland samples, and 52.9% of labial salivary gland samples [28]. The authors demonstrated that the salivary glands are the site of constant presence of HHV6.

The study of the number of HHV6 DNA copies was carried out in saliva samples using the polymerase chain reaction (PCR) method with real-time hybridization-fluorescence detection. The test systems “AmpliSens EBV/CMV/HHV6-screen-FL” of the Central Research Institute of Epidemiology (Russia) were used. To measure the amount of DNA in saliva, we use the number of copies of HHV6 DNA in 1 ml of the sample (cDNA). This indicator is calculated using the formula: CDNA = CDNA x 100, where CDNA is the number of copies of viral DNA in the sample. The analytical sensitivity of the test system is 400 copies/ml (according to the manufacturer's instructions).

Interleukin-1β (IL-1β) Production in Culture Medium (Spontaneous and Induced Production) and Serum

Peripheral blood was collected from the cubital vein into a vacutainer containing a coagulation activator (to obtain serum) and into a vacutainer containing lithium heparin as an anticoagulant (for culture studies). To obtain serum, blood was centrifuged at 3000 rpm/10 min.

To obtain cytokines, peripheral blood cells were stimulated with pyrogenal (N.F. Gamaleya National Research Center of Epidemiology and Microbiology, Ministry of Health of the Russian Federation). Pyrogenal was added at a working dilution of 10 μg/ml per well and incubated with whole blood for 24 hours in the presence of carbon dioxide at 37°C. IL-1β levels in serum and supernatant were determined by enzyme-linked immunosorbent assay using the Interleukin-1 beta-ELISA-Best test system (Vector Best, Russia).

Statistical analysis of the obtained results was performed using the IBM SPSS Statistics software package, version 26 (Armonk, NY: IBM Corp.). Group results are presented as the arithmetic mean ± standard error (M ± SD). For a comparative assessment of the results in the patient groups, the nonparametric Mann-Whitney U-test was used. To assess the probability of differences between groups, the Student's t-test was used. Differences

between groups were considered significant at $p \leq 0.05$. To verify compliance with the condition of independence of observations, we performed linear regression analysis (with calculation of the coefficient of determination (R-squared) and the Durban-Watson criterion) and analysis of variance (ANOVA) with calculation of the Fisher criterion (F) to test the significance of the model. The standardized β value with 95% confidence intervals was calculated. The critical significance level for differences in the indicators was set to 0.05.

Results

Cognitive Complaints in Patients with Chronic HHV-6 Infection

For adults, primary infection is not as typical as for children and is rare. Typically, adult patients experience reactivation of the infection, which leads to the development of clinical complaints. The most typical complaints include undifferentiated fever or mononucleosis-like syndrome, atypical lymphocytosis, and itching [29]. Patients also often develop multiple neurological complaints, weakness, alopecia, fatigue, decreased hearing acuity, and gastrointestinal and respiratory symptoms (bronchitis, tracheitis) [30]. Patients are typically exposed to prolonged stress, psychoemotional and physical overload, which leads to the progression of complaints. In particular, the work of Bronzuoli MR et al. showed that reactivation of herpesvirus infection may be associated with deterioration of cognitive function in patients [31].

The Content of IgG Antibodies to HHV6 in the Blood Serum (IgG-HHV6)

The content of antibodies in the serum of patients was determined during examination by an infectious disease specialist. The patients provided the obtained results when visiting for a consultation. The level of antibodies in patients was not re-examined. The content of IgG-HHV6 was 4.20 ± 0.29 (95% CI: 3.65 - 4.78): in men it was 4.45 ± 0.71 (95% CI: 3.11 - 5.92), in women it was 4.17 ± 0.31 (95% CI: 3.58 - 4.84) (p = 0.690). An analysis of the content of IgG-HHV6 was performed in patients with severe or mild cognitive complaints (Table 1).

Table 1: The content of IgG-HHV6 in the blood serum of patients with and without cognitive complaints.

Cognitive complaints	Patient group (n=66) Complaint frequency (%)	Serum IgG-HHV6 (PC*) (with/without complaints)	P
Impaired concentration	78,78 (52/66)	3,99±0,34 (95% CI:3,32 – 4,68) 4,54± 0,54 (95% CI: 3,52 – 5,64)	0,357
Memory impairment	74,24 (48/66)	4,10±0,32 (95% CI: 3,48 – 4,76) 4,46±0,61 (95% CI:3,33 – 5,76)	0,587
Headaches	68,128 (45/66)	4,21±0,34 (95% CI: 3,55 – 4,90) 4,20±0,55 (95% CI: 3,21 – 5,28)	0,983
Sleep disturbances	69,69 (46/66)	4,35±0,33 (95% CI: 3,70 – 5,08) 3,83±0,54 (95% CI:2,78 – 4,90)	0,413
Panic attacks	53,03 (35/66)	4,31±0,37 (95% CI: 3,61 – 5,05) 4,08±0,45 (95% CI: 3,26 – 5,03)	0,704
Unmotivated irritability	74,24 (48/66)	4,12±0,32 (95% CI: 3,51 – 4,77) 4,45±0,60 (95% CI:3,33 -5,63)	0,621
Tearfulness	84,84 (55/66)	4,06±0,30 (95% CI: 3,45 – 4,64) 4,98±0,78 (95% CI: 3,41 -5,65)	0,260
Fatigue	78,78 (52/66)	4,38±0,37 (95% CI: 3,65 – 5,11) 3,75±0,42 (95% CI:2,88 – 4,57)	0,257

* PC - positivity coefficient

The data in the table show that IgG-HHV6 are present in the blood serum of all patients, regardless of the severity of cognitive complaints.

HHV-6 DNA Copy Number in Saliva Samples and the Development of Cognitive Complaints in Patients

All patients underwent PCR testing for HHV6 DNA copy number in saliva samples. The DNA copy number was 1114.24±180.27 (95% CI: 767.74 - 1491.17). It should be noted that the HHV6 DNA copy number in 1 milliliter of saliva ranged from 10^2 to 10^3 (range: 110 to 8200). No patient had a DNA copy number of 10^4 or more (Figure 2). In women, the HHV6 DNA copy number was -918.00±252.01 (95% CI: 575.54 -1483.39), while in men, the DNA copy number was significantly higher: 1302.53±428.88 (95% CI: 568.11 - 2234.98) (p = 0.010).

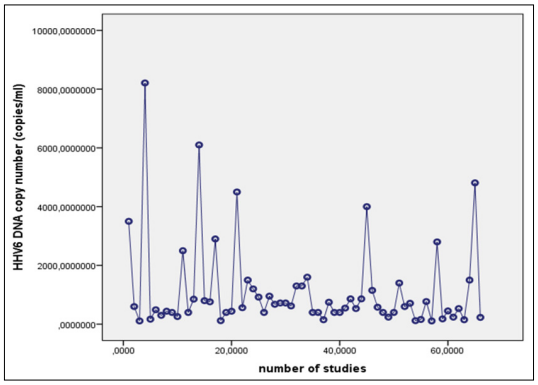

Figure 2: HHV6 DNA copy number content in saliva samples from patients.

A regression analysis revealed an association between HHV-6 DNA copy number and patient complaints. The results are presented in Table 2.

Table 2: Regression model of the relationship between the number of HHV-6 DNA copies and the development of cognitive complaints in patients with chronic HHV-6 infection.

Indicator	Equation	Determination coefficient (R2)	Fisher criterion (F)	Standardized coefficient (β)	P
Impaired concentration	Linear Logistic	0,275 0,610	24,598 100,107	0,524 0,458	0,000 0,000
Memory impairment	Linear Logistic	0,252 0,674	21,940 132,028	0,502 0,404	0,000 0,000
Headaches	Linear Logistic	0,325 0,645	40,169 116,398	0,618 0,448	0,000 0,000
Sleep disturbances	Linear Logistic	0,209 0,712	17,193 150,651	0,457 0,433	0,000 0,000
Panic attacks	Linear Logistic	0,163 0,516	12,432 68,195	0,403 0,488	0,001 0,000
Fatigue	Linear Logistic	0,327 0,683	313519 137,976	0,570 0,438	0,000 0,000

Regression analysis demonstrated an association between induced IL-1β and the development of cognitive complaints in patients.

When conducting an «Analysis of variance» (ANOVA) to test the significance of the resulting model, significant results were obtained between the HHV-6 DNA copy number:

- With memory loss: F=19.060 β=0.479 p=0.000;
- With impaired concentration: F=21.310 β=0.500 p=0.000;
- With sleep disturbance: F=17.434 β=0.463 p=0.000;
- With headaches: F=11.805 β=0.395 p=0.001;
- With fatigue: F=21.241 β=0.499 p=0.000;
- With panic attacks: F=12.432 β=0.403 p=0.001.

Interleukin-1β (IL-1β) Levels in Culture Medium and Serum

The obtained IL-1β levels (serum, spontaneous, and induced) are presented in Table 3.

Table 3: IL-1β production in serum and culture medium in patients with chronic HHV6 infection (n=66)

Parameter	IL-1β level (pg/ml)	Reference values (pg/ml)
Serum level	1.64±0.29 95% CI: 1.17-2.31	0 - 11
Spontaneous level	13.60±3.83 95% CI: 6.87 -22.32	1 - 107
Induced level	2383.03±277.90 95% CI: 1909.35 -2985.25	50 - 1200

As can be seen from the table, serum and spontaneous IL-1β production values were stable and did not differ from reference values. Induced IL-1β levels were higher than the reference values (50–1200 pg/ml, reference values provided by the test system manufacturer). The mean induced IL-1β level in the patient group exceeded the upper reference value by 1,98 times. Induced IL-1β production is shown in Figure 3.

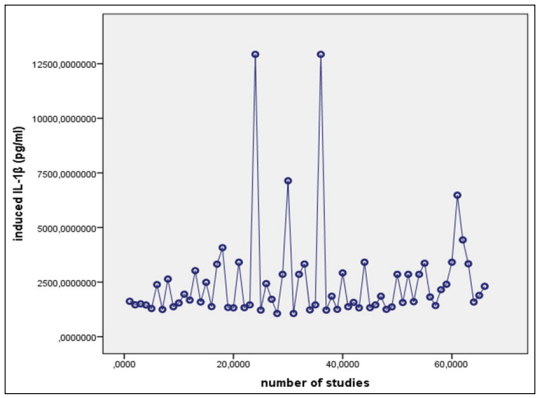

Figure 3: Level of induced IL-1β production in the culture medium of patients with HHV6 infection in the general group.

Next, a regression analysis was performed between induced IL-1β levels and medical history data (age, disease duration, depression, stress, antidepressant use, and HHV6 DNA copy number in saliva samples). The results are presented in Table 4.

Table 4: Regression Model of The Relationship Between the Level of Induced Il-1β And Anamnesis Data

Indicator	Equation	Determination coefficient (R2)	Fisher criterion (F)	Standardized coefficient (β)	P
Age	Linear Logistic	0,484 0,915	60,138 692,223	0,696 0,384	0,000 0,000
Disease duration	Linear Logistic	0,531 0,852	72,320 367,432	0,728 0,397	0,000 0,000
HHV6 DNA copy number	Linear Logistic	0,168 0,354	12,948 35,054	0,410 0,552	0,001 0,000
Depression	Linear Logistic	0,215 0,379	17,494 38,982	0,463 0,541	0,000 0,000
Stress	Linear Logistic	0,551 0,855	78,615 376,193	0,742 0,397	0,000 0,000
Antidepressant use	Linear Logistic	0,526 0,859	70,945 390,310	0,725 0,396	0,000 0,000

An «Analysis of variance» (ANOVA) with the F test to test the significance of the model showed that the resulting model was significant:

• Induced IL-1β levels and age: F = 68.842 β = 0.707 p = 0.000;
• Induced IL-1β levels and disease duration: F = 78.519 β = 0.739 p = 0.000;
• Induced IL-1β levels and HHV6 DNA copy number: F = 72.320 β = 0.728 p = 0.000.
• Induced IL-1β levels and depression: F=8.044 β= 0.325 p=0.006;
• Induced IL-1β levels and stress development: F=86.420 β= 0.748 p=0.000;
• Induced IL-1β levels and antidepressant use: F=79.884 β=0.735 p=0.000;

These results confirmed the significance of the resulting model.

Production of induced IL-1β in Culture Medium and the Development of Cognitive Complaints in Patients with Chronic HHV-6 Infection.

Linear and logistic regression analyses revealed significant associations between cognitive complaints and the level of induced IL-1β production in the culture medium (Table 5).

The table shows that induced IL-1β levels are associated with the development of cognitive complaints. When conducting an ANOVA to test the significance of the resulting model, the following results were obtained. Induced IL-1β levels are associated with:

- Memory impairment: F=42.660 β=0.632 p=0.000;
- Attention impairment: F=18.610 β=0.475 p=0.000;
- Sleep disturbance: F=59.295 β=0.693 p=0.000;
- Headaches: F=68.861 β=0.712. p=0.000;
- With fatigue: F=42.660 β=0.632 p=0.000;
- With panic attacks: F=29.303 β=0.560 p=0.000.

These results indicate that the level of induced IL-1β is associated with the development of cognitive complaints in patients, as statistically significant results were obtained.

Table 5: Regression model of induced IL-1β levels and cognitive complaints in patients with chronic HHV-6 infection

Indicator	Equation	Determination coefficient (R2)	Fisher criterion (F)	Standardized coefficient (β)	P
Impaired concentration	Linear Logistic	0,451 0,628	40,203 108,059	0,671 0,453	0,000 0,000
Memory impairment	Linear Logistic	0,419 0,722	46,159 165,908	0,647 0,428	0,000 0,000
Headaches	Linear Logistic	0,382 0,507	40,169 65,861	0,618 0,712	0,000 0,000
Sleep disturbances	Linear Logistic	0,447 0,730	39,537 173,216	0,668 0,425	0,000 0,000
Panic attacks	Linear Logistic	0,350 0,537	26,375 74,252	0,592 0,481	0,000 0,000
Fatigue	Linear Logistic	0,537 0,717	75,365 161,788	0,733 0,429	0,000 0,001

Discussion

In 1990, R. Schindler et al. published the results of a study examining the effect of HHV6 on the production of proinflammatory cytokines IL-1α, IL-1β, TNFα, and IL-6 by peripheral blood mononuclear cells in 38 donors [32]. Maximum cytokine production was detected 20–24 hours after infection, with a subsequent decrease in the production of these cytokines. In 1991, Flamand L. et al. published the results of a study on the production of IL-1β and TNFα in patients infected with HHV6 and showed that maximum IL-1β production (2500 pg/mL) was observed during the first 48 hours after infection of cells, with a decrease in production beginning in the following days [33]. Uninfected cells produced 600 pg/ml IL-1β. The authors concluded that HHV-6 is a potent inducer of IL-1β. Polymerase chain reaction revealed maximum transcription of the IL-1β and TNF-α genes 12 hours after cell infection. To clarify the mechanism of proinflammatory cytokine induction, mononuclear cells were treated with phosphoacetic acid (an inhibitor of viral DNA polymerase activity), which effectively inhibits the replication of the viral cationic protein.

Tetsushi Yoshikawa et al. showed that an increase in IL-1β production was detected during reactivation of HHV-6 infection [34].

We analyzed the production of the proinflammatory cytokine IL-1β in patients with reactivation of chronic HHV6 infection. Our results demonstrated that all patients (n=66) showed increased production of induced IL-1β. Regression analysis yielded both linear and logistic regression between the level of induced IL-1β and the number of HHV6 DNA copies in their saliva samples (p=0.001 and p=0.000), which was confirmed by analysis of variance (p=0.001). Thus, it can be assumed that induced IL-1β production is associated with the number of HHV6 DNA copies.

Regression analysis showed a relationship between induced IL-1β, patient age, and disease duration. This relationship was confirmed by analysis of variance (p=0.000). Thus, it can be hypothesized that in the study of patients with chronic infection, the increased production of IL-1β by blood mononuclear cells is associated with the duration of the patient's illness. Previously, Megan E. Roerink et al. showed that the duration of the disease affects the level of IL-1β production, i.e., with a short duration of the disease, the level is increased, and with a long duration of the disease, it is decreased [35]. In the present study, it was not possible to conduct this analysis, since there were no patients with an acute stage in the study, and we do not know the baseline level of IL-1β.

Patients included in the study were referred by an infectious disease specialist. It should be noted that the severity of cognitive impairment was noted during the analysis of patient complaints. In our work, it was shown that HHV-6 infection itself was associated with cognitive complaints in patients. This was confirmed by analysis of variance.

During the anamnesis collection, when we asked: "What preceded the onset of your complaints and deterioration of your condition?" patients unambiguously indicated "chronic depression/chronic stress". All of our patients with chronic HHV-6 in the study were observed by a psychiatrist and took antidepressants (drugs of the selective serotonin reuptake inhibitor group) for a long time. IL-1β plays a major role in the development of depression and stress, as it reduces the reuptake of serotonin from the synaptic cleft and is a potent regulator of the expression of the serotonin transporter gene in humans. Thomas A.J., et al. in their study demonstrated an increase in IL-1β levels in patients over 60 years of age, which correlated with the severity of depression [36]. In the study group, all patients with depression and prolonged stress had elevated levels of induced IL-1β. Our study also showed an association between IL-1β levels, depression, stress and antidepressant use using logistic regression analysis and ANOVA analysis of variance, which is consistent with previously published data by other authors.

In 2008, Koo JW et al. demonstrated for the first time in their work that the antineurogenic effects of acute and chronic stress are due to the action of IL-1β, which reduces astrocyte proliferation by arresting the cell cycle as a result of activation of the NF-κB pathway in astrocytes [37]. In an experiment on male Sprague Dawley rats, Meghan E. Jones et al. demonstrated that during stress, it is astrocytes that are the cellular source of IL-1β in the hippocampus, causing important neuronal changes [38]. As a result of peripheral stimulation of the immune system, IL-1β is stably produced in the brain. Physiological levels of IL-1β are necessary for normal memory function, and excessively low or high concentrations of the cytokine lead to memory impairment and the development of neurodegeneration [39]. This suggests that IL-1β plays a significant role in the formation of hippocampus-dependent memory. Inbal Goshen et al. demonstrated that IL-1β's involvement in hippocampal-dependent memory processes follows an inverted-U-shaped curve.

This suggests that a modest increase in IL-1β production in the brain improves memory, while an excessive increase in IL-1β levels or blockade of IL-1β signaling leads to memory impairment [40]. In our study, linear and logistic regression methods were used to show an association between the level of induced IL-1β and the severity of memory loss in patients (R2=0.484 β=0.696 F=45.929 p=0.000; R2=0.722 β=0.428 F=165.908 p=0.000, respectively). This was also confirmed by analysis of variance (F=42.660 β=0.632 p=0.000). The obtained results can be explained by the results of a study published earlier by Inbal Goshen et al., which showed that an increase in the level of IL-1β leads to memory impairment [40].

IL-1β plays a significant role in the development of neuroinflammation and is secreted during depression and anxiety, thus serving as a biomarker of stress and sleep disturbances [41]. Chronic neuroinflammation leads to the development of symptoms such as fatigue, cognitive decline, increased sensitivity to pain and stimuli, and excessive sleepiness. Krueger, J. M., and others suggested in their work that IL-1β plays a role in the regulation of sleep. This relationship was confirmed in the study conducted in our patients by the obtained linear and logistic regression between the level of induced IL-1β and sleep disturbance in patients (R2=0.447 β=0.668 F=39.537 p=0.000; R2=0.730 β=0.668 F=173.216 p=0.000, respectively). Confirmation was obtained by the analysis of variance method (F=59.295 β=0.693 p=0.000). Krueger J.M. also proposed to

consider IL-1β as a substance that promotes the development of the slow sleep phase under physiological and inflammatory conditions [42]. The association between IL-1β production and the development of panic disorders was first described by Brambilla F. in their study [43]. This relationship was subsequently studied in patients with Parkinson's disease. An elevated level of IL-1β was identified, which is associated with changes in the activity of the hypothalamic-pituitary-adrenal system, activation of microglia, tryptophan metabolism, and an imbalance of excitatory and inhibitory neurotransmission. Thus, the authors of the study associated current panic disorder with higher levels of inflammatory cytokines, such as IL-6 and IL-1β, compared to the control group [44]. In the group of patients with chronic HHV-6 infection, the incidence of panic attacks is 53.3%. Linear and logistic regression were found to associate the level of induced IL-1β with the development of panic attacks (R2=0.350 β=0.592 F=26.375 p=0.000; R2=0.537 β=0.481 F=74.252 p=0.000, respectively). ANOVA analysis of variance confirmed this association (F=29.303 β=0.560 p=0.000). Thus, it can be assumed that elevated levels of induced IL-1β are associated with panic attacks in patients with chronic HHV-6 infection.

The central nervous system plays a crucial role in the perception of fatigue, as it processes and evaluates sensory information and controls motivational behavior associated with decisions to stop an activity or to apply effort. It has been shown that increases in pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF, as well as IFN-1, in the brain lead to behavioral changes described as "sickness behavior". This concept includes increased fatigue, depressed mood, loss of interest in social interaction, and decreased physical activity. Changes in patient behavior associated with infection are the result of the interaction of proinflammatory cytokines with neurons [45]. Clinical studies confirm that fatigue is caused by an inflammatory process, that is, the induction of cytokines in healthy people causes a feeling of fatigue. According to Vollmer-Conna U. et al., during the acute infectious period, a decrease in IL-1β production by peripheral blood cells demonstrated an insignificant correlation with fatigue symptoms [46]. During the chronic course of infectious symptoms, this correlation was absent [47]. In the study, the linear regression method was used to show the influence of induced IL-1β on the severity of fatigue development in patients with chronic infection (R=0.537 F=75.365 β=0.733 p=0.000; R=0.717 F=161.788 β=0.429 p=0.001, respectively). ANOVA analysis of variance confirmed the presence of a relationship with fatigue (F=42.660 β=0.632 p=0.000). Patients describe fatigue as a manifestation of cognitive and emotional impairments, and decreased motor activity. This description is absolutely consistent with the definition of fatigue given by Kaster M. P. in their work [48]. 66 patients participated in the study. Fatigue was present in 78.78% of patients, but no association with gender or age was found. This was also noted in the work of Medvedev A. E. [49,50].

Conclusion

Cytokines are key signaling molecules produced by immune and non-immune cells that affect the periphery and brain and are key regulators of acute and chronic inflammation [50]. This study examined the production of IL-1β in patients with reactivation of chronic HHV-6 infection. Based on the results, it can be suggested that the production of induced IL-1β is associated with cognitive complaints in the patients examined.

Furthermore, it can be hypothesized that the number of HHV-6 DNA copies is associated with increased IL-1β production. However, we cannot confirm that this is specific to this group of patients, as it is impossible to conduct a comparative analysis with other groups.

Limitations

This study has several limitations that must be considered. First, the lack of a control group is a limitation of the study. Healthy individuals could serve as a control group. However, they never seek consultations or immunological testing at a medical facility. We do not have a group of patients with latent chronic HHV-6 infection, as neither general practitioners nor infectious disease specialists refer these patients to an immunologist for examination and treatment unless they have complaints. The lack of such patient groups for comparative analysis with patients with reactivated chronic HHV-6 infection is a limitation of the study and does not allow for a comparative analysis between the different patient groups. Therefore, we do not draw definitive conclusions, but rather offer hypotheses.

Secondly, other specialists initially examined the patients, and therefore we had no way to influence the scope of the examination. For example, during the neurologist examination, the Mini-Mental State Examination (MMSE) was used, although we acknowledge that other methods exist.

Future research directions

The obtained results, namely the association between increased IL-1β and cognitive complaints in patients with chronic VHH6 infection, clearly require further study involving the assessment of other pro-inflammatory cytokines. It would be interesting to study this issue in patients with other herpesvirus infections, such as Epstein-Barr virus infection and cytomegalovirus infection. Also of great interest is the study of the subpopulation composition of these patients. This is planned for future studies and comparison with a control group.

Data Sharing Policy

The statistical code, dataset used in support of the findings of this study are included within the article.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Authors' contribution

Study concept and research design, statistical processing of data, and research supervision as well as responsibility for integrity of all parts of the article (RIA); material gathering and processing, data analysis and interpretation, and script composition (RIA and RTS); lab research (KAA); diting (RTS and KAA); and

text writing and editing as well as further revision for important intellectual content (RIA, RTS, and KAA). All authors have made substantial contributions to this study and approved the final version to be published.

All the authors have made substantial contribution to this study and approved final script version.

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