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Research Article

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Treatment of Chronic Reactivation HHV-6 Infection with Valganciclovir

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ABSTRACT

Research Objective: Currently, there are no officially approved antiviral drugs used for the treatment of chronic herpes virus type 6 (HHV-6). Ganciclovir, a nucleoside analog synthesized in 1980 by Kelvin Kenneth Ogilvie and patented for the treatment of cytomegalovirus infection, is widely studied and used for the treatment of HHV-6 infection. In 1988, the drug was approved for medical use. Among all antiviral drugs discovered to date, ganciclovir has the strongest inhibitory effect on the activity of HHV-6.

Aim: The research was to study the effectiveness of valganciclovir in the treatment of patients with reactivation of HHV-6 infection.

Material and Methods: The study was conducted in 50 patients with reactivated chronic HHV-6 infection. The ratio of women to men was 3:1 (women – 37, men – 13). The average age of the patients was 34.12 ±1.24 years (95% CI: 31.68 - 36.67). The duration of the disease was 2.57 ± 0.15 years (95% CI: 2.28 – 2.90). HHV-6 infection was confirmed by the presence of HHV6 DNA copies in saliva samples, the presence of IgG antibodies to HHV6 in the serum, and severe clinical symptoms. All patients received valganciclovir therapy at a daily dose of 900 mg for 6 months. To assess the effectiveness of therapy, HHV-6 DNA copy number analysis in saliva samples, IFN-α and IFN-γ production levels, and changes in clinical symptoms were performed monthly.

Results: A significant reduction in DNA copy number was demonstrated after three months, by 39.62% of the baseline HHV6 DNA copy number (from 1175.40 ± 224.23 to 709.72 ± 104.84 copies/ml, p = 0.001), and after six months, by 53.45% (from 1175.40 ± 224.23 to 547.20 ± 74.88 copies/ml, p = 0.001). A decrease in induced IFN-α production and normal levels of serum, spontaneous, and induced IFN-γ were detected. After completion of valganciclovir therapy, all patients continued to experience some isolated complaints. However, therapy was discontinued after 6 months, as the patients' condition became stable by the end of the fifth to the beginning of the sixth month of therapy. The majority of patients (72% (36/50)) rated their condition by the end of therapy as "satisfactory," and 28% (14/50) as "good," without any significant complaints. Patients rated the effect of the treatment as "positive."

Conclusion: Long-term valganciclovir therapy for chronic HHV6 infection significantly reduces the number of DNA copies in saliva samples, but does not completely eliminate the DNA copy number. A significant reduction in all clinical complaints was demonstrated after 6 months of therapy.

Keywords: Antiviral Therapy, Valganciclovir, Herpes Virus Type 6, DNA Copy Number

The discovery of a new human herpesvirus was first reported in 1986 [1]. The virus was isolated from interleukin-2-stimulated peripheral blood mononuclear cells of patients with lymphoproliferative diseases and AIDS. Electron microscopy revealed the morphology of the viral particles, which was identical to that of herpesviruses [2]. The virus consists of a capsid of icosahedral symmetry, surrounded by a tegument, covered by an envelope with a diameter of about 200 nm [3]. The virus was initially named "human B-lymphotropic virus (HBLV)", but was soon identified as a T-lymphotropic virus and named "human herpesvirus 6" (HHV6) (strain GS) and officially classified in the order Herpesvirales, family Herpesviridae subfamily Betaherpesvirinae, genus Roseolovirus [4,5]. In 1987 and 1988, independent isolates designated U1102 were isolated from AIDS patients in Uganda, Africa, and Z29 in Zaire [6-8]. Z-29 type HHV-6 isolates from peripheral blood mononuclear cells of patients with roseola, bone marrow transplant recipients, leukopenic patients, and "an HIV-1-positive" AIDS patient from Zaire were shown to be closely related to but distinct from GS

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type HHV-6 isolates. The resulting HHV-6 isolates proliferated in T cells (CEM, H9, Jurkat), monocytes (HL60, U937), glial cells (HED), and B cells (Raji, RAMOS, L4, WHPT) [7,8]. T cells in which the virus undergoes its life cycle have been identified as CD4+ T lymphocytes [9]. Subsequently, based on the obtained data, HHV-6 isolates were divided into group A (GS type) and group B (Z-29 type), that is, the double-stranded DNA-containing HHV-6 virus includes two types: HHV-6A and HHV-6B [2,10,11]. HHV-6A has greater virulence and cytotoxicity, while HHV-6B is the causative agent of sudden rash in roseola, a disease characterized by high fever and skin rash that occurs in childhood [12]. Under experimental conditions, it was shown that these two HHV-6 groups had different in vitro tropism for T-cell lines, different patterns of endonuclease restriction sites, and specific interstrain variations in DNA sequences [13]. In 1992, the scientific community suggested that these two HHV-6 groups (HHV-6A and HHV-6B) were variants of the same species [14]. In 2010, an Ad Hoc Committee on HHV-6A & HHV-6B Genomic Divergence submitted a proposal to the International Committee of Taxonomy of Viruses (ICTV) to recognize HHV-6A and HHV-6B as separate viruses. In 2012, the International Committee on Taxonomy of Viruses officially classified HHV-6A and HHV-6B as separate viruses, replacing the species human herpesvirus 6 with human herpesvirus 6A and human herpesvirus 6B in the genus Roseolovirus, subfamily Betaherpesvirinae, family Herpesviridae, order Herpesvirales. Human herpesvirus 6A was recognized as the type species in this genus [15]. Both viruses have different epidemiological and biological characteristics and clinical manifestations [16].

Currently, there are no precise data on the prevalence of infections caused by HHV-6A and HHV-6B, as serological methods do not allow for differentiation between these infections. Therefore, the literature lacks precise data on the prevalence of infection in various population groups in different countries. These results also depend on the age of the subjects, the geographic location, and the sensitivity and specificity of serological tests. According to the study, the prevalence of infection of children in the Zambia region with HHV-6A is less than 1%, and HHV-6B is approximately 20% among hospitalized children under two years of age. Most newborns receive passive immunity to HHV6 from their mother. Infection of the child occurs when the mother's antibody levels to the virus decrease. Pruksananonda P., et al. in their work showed that in children under 1 month of age, infection is 10%, and at 12 months of age – 66% [2,17-19].

Zerr D.M. and colleagues published the results of a prospective study of 277 children aged between birth and two years, aimed at identifying the pattern of HHV-6 infection. For this purpose, the children's saliva samples were tested weekly using polymerase chain reaction for the presence of HHV-6 DNA copies. The peak infection rate was 40% at 12 months, and 77% at 24 months. Peak infection rates were recorded between the ages of 9 and 21 months. Seroconversion occurs between the ages of 1 and 3 years, indicating an oral route of infection. By the age of 40, infection rates range from 80% to 100%, and among people aged 62 to 88 years, infection rates decrease to 35%. Following primary infection, persistent infection develops, with the formation of a lifelong latent phase, with the viral genome persisting in peripheral blood mononuclear cells (PBMCs), endothelium, monocytes/macrophages, dendritic cells, salivary glands, and brain tissue [20-25].

Latency is the lifelong presence of the virus in a modified form in host cells. Hudnall SD, et al. published the results of a study using real-time polymerase chain reaction to detect the viral load of DNA of all herpesviruses in blood mononuclear cells conducted in 100 randomly selected blood donors from the southeastern region of Texas. The authors showed that HHV-6B was detected in 30% of healthy individuals. Only one healthy individual had more than 6.1 x 10^7 copies of HHV-6B DNA per milliliter of blood in a peripheral blood sample, which was not associated with an active infection or immunodeficiency. During the persistence period, the virus continuously or cyclically replicates in infected cells, leading to the development of the infectious process. Henri Agut et al. demonstrated in their work that the main route of infection with the virus is contact with the saliva of an infected person, as demonstrated by the presence of the virus in saliva and salivary glands. Currently no officially approved antiviral drugs exist for the treatment of HHV-6 infection. The development of drugs that specifically target HHV-6 remains a relatively low priority. Broad-spectrum antiviral drugs, including those against roseolaviruses, remain the most effective. Ganciclovir, a nucleoside analogue, is being widely studied and used for the treatment of HHV-6 infection. In 1980, Kelvin Kenneth Ogilvie synthesized and patented the antiviral drug ganciclovir, a nucleoside analogue, for the treatment of cytomegalovirus (CMV) infection. In 1988, the drug was approved for medical use [2,26-29].

Ganciclovir is a broad-spectrum antiviral drug that can block the synthesis of the viral DNA chain, suppress the activity of DNA polymerase and reverse transcriptase, and inhibits viral replication and proliferation. It has a rapid effect and a long duration of action, and is therefore widely used in clinical practice. Stockmann C. et al. Concluded that among all antiviral drugs discovered to date, ganciclovir has the strongest inhibitory effect on viral activity. The primary mechanism of action is that ganciclovir can be converted to active ganciclovir triphosphate by deoxyguanosine kinase in infected cells, resulting in competitive inhibition of the binding of deoxyguanosine triphosphate to viral DNA polymerase, terminating the elongation of the viral DNA chain. This effect was demonstrated most strongly in infected cells, indicating a potent antiviral activity. Ganciclovir is phosphorylated by HHV-6 U69 kinase, and the active triphosphate metabolite inhibits DNA polymerase [28,30-32].

HHV-6 resistance to ganciclovir is related to both U69 protein kinase and U38 DNA polymerase. Based on the published results of Leen De Bolle et al., it was concluded that the pU69 M(318)V amino acid substitution, rather than the A(961)V substitution in the DNA polymerase of human herpes virus type 6, is responsible for the ganciclovir resistance phenotype [33]. The mechanisms underlying the development of resistance to HHV-6 include:

1. active viral replication;
2. prolonged drug exposure;
3. the use of suboptimal doses.

Leen De Bolle et al. demonstrated that the HHV-6 U69 gene product (pU69) is expressed as a nuclear protein and may be a functional homolog of human cytomegalovirus UL97 kinase

(pUL97), which converts ganciclovir to its monophosphate metabolite in CMV-infected cells. pU69 is able to limit ganciclovir phosphorylation, contributing to the moderate antiviral activity of ganciclovir against HHV-6. Similar data on the effect of HHV-6 U69 mutations that provide resistance to ganciclovir, similar to mutations in human CMV UL97 protein kinase that cause resistance to ganciclovir, were published by Safronetz, D., et al. The risk of developing HHV-6 resistance to valganciclovir exists and has been described in the literature [34-36].

Aim of this study is to investigate the efficacy of valganciclovir in the treatment of patients with HHV-6 reactivation.

Material and Methods

Fifty patients with reactivation of chronic HHV-6 infection were included in a single-center cohort prospective study. The female to male ratio was 3:1 (37 women, 13 men). The mean age of patients was 34.12 ± 1.24 years (95% CI: 31.68 - 36.67). The duration of the disease was 2.57 ± 0.15 years (95% CI: 2.28 - 2.90). Before initiating antiviral therapy, patients with clinical complaints were examined by a general practitioner, neurologist, otolaryngologist, and rheumatologist. No pathology was detected. Subsequently, the patients were examined by an infectious disease specialist, where chronic herpesvirus infection associated with the HHV-6 virus was diagnosed. This was confirmed by the presence of HHV6 DNA copies in saliva samples, IgG antibodies to HHV-6 in the serum, and severe clinical complaints. No HHV-6 DNA copies were detected in peripheral blood mononuclear cells. No patient was diagnosed with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) by a neurologist. An infectious disease specialist assessed laboratory parameters and clinical complaints as manifestations of reactivation of chronic HHV6 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 before starting therapy;
- Presence of clinical complaints caused by HHV6;
- Signed informed consent prior to the study.

Exclusion Criteria Included the Presence of:

- Other viral infections (cytomegalovirus infection, viral hepatitis, human immunodeficiency virus).
- Toxoplasmosis, borreliosis, staphylococcal and streptococcal infections.
- Helminthic infestations, parasitic infections.
- Acute inflammatory diseases of any organs.
- Mental illness.
- Aautoimmune diseases.
- Use of any antiviral medications within the past 6 months.
- Pregnancy in women.
- Regular alcohol consumption.

The clinical study was conducted in compliance 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) and was approved by the local Ethics Committee of the St. Petersburg Dialysis Center LLC FRESENIUS MEDICAL CARE (protocol No. 9 dated 12.02.2023).

Each patient's medical and lifestyle history was collected, including any previous courses of antiviral therapy. The patients' clinical condition was assessed using a standard method, including objective data and the patient's 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. All patients received valganciclovir therapy at a daily dose of 900 mg for at least 6 months. Valganciclovir was well tolerated by patients and did not cause hepato-nephrotoxicity or hematopoietic toxicity, as monitored monthly by blood tests for ALT, AST, bilirubin, creatinine with calculated glomerular filtration rate (GFR), cystatin with calculated GFR, and complete blood count. The drug also did not cause any allergic reactions. To assess the effectiveness of therapy, monthly analyses were performed to measure the HHV-6 DNA copy number in saliva samples, the level of serum, spontaneous, and induced IFN-α and IFN-γ production in lymphocyte cultures, and the dynamics of clinical complaints.The literature shows that in chronic and atypical forms of herpesvirus infection, analysis of saliva samples is more informative for determining the number of copies of viral DNA [37].

Detection of Viral DNA by PCR in Saliva Samples

The assay used in this study does not differentiate HHV-6a from HHV-6b. Precise identification of the HHV-6 pathogen currently has no impact on diagnosis or treatment. 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 (CNDNA). This indicator is calculated using the formula: CNDNA = 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).

The Serum Levels of IFN-α and IFN-γ, As well as the Spontaneous and Induced Production of these Cytokines in Blood Lymphocyte Cultures, were Determined

Newcastle disease virus (NDV) (obtained at the Scientific Centre for Expert Evaluation of Medicinal Products of the Ministry of Health of the Russian Federation, Moscow, Russia) with an infectious titer of 8 lg EID/0.2 ml in a volume of 8 µl per well was used as an IFN-α inducer. Phytohemagglutinin (PHA-P) (PanEco, Russia) at a dose of 10 µg/ml was used as an IFN-γ inducer. Cytokine levels were determined in serum and supernatant of 24-hour whole blood cultures using solid-phase ELISA using the alpha-Interferon-IFA-BEST and gamma-Interferon-IFA-BEST test systems (Vector Best, Russia). Reference values for spontaneous, serum, and induced IFN-α and IFN-γ production were provided by the test system manufacturer.

Statistical Analysis

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). The nonparametric Mann–Whitney U-test was used to compare results within patient groups. The Student's t-test was used to assess the probability of differences between groups. Differences between groups were considered significant at $p \leq 0.05$.

Results

All patients underwent PCR testing for HHV6 DNA copy number in saliva samples. Before initiation of valganciclovir therapy, the DNA copy number was 1175.40 ± 219.71 (95% CI: 810.97 - 1653.64). It should be noted that the HHV6 DNA copy number in 1 milliliter of saliva ranged from 10^2-10^3 (range 110 to 8200). No patient had a DNA copy number of 10 to the 4rd power or more (Figure 1).

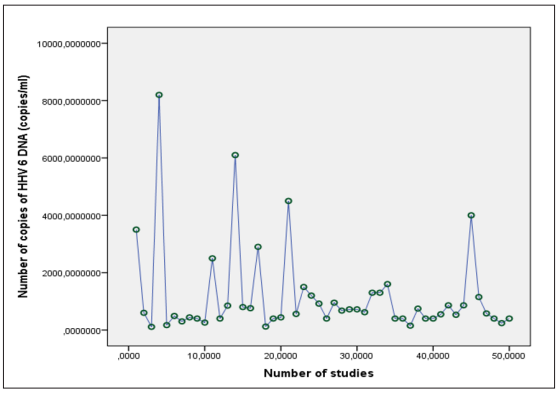

Figure1: HHV6 DNA copy number content in the patient group before therapy.

In women, the HHV6 DNA copy number was 1052.42 ± 239.99 (95% CI: 661.69 - 1599.46), while in men, the DNA copy number was significantly higher: 1475.23 ± 462.06 (95% CI: 687.01 - 2447.75) (p = 0.011).

Dynamics of HHV6 DNA Copy Number During Valganciclovir Therapy

The dynamics of HHV6 DNA copy number in saliva samples were assessed monthly during therapy throughout the entire course of valganciclovir therapy (Table 1).

Table 1: Dynamics of HHV6 DNA Copy Number in Saliva Samples in Patients Receiving Valganciclovir Therapy

Time of study	Number of HHV6 DNA copies in a saliva sample (copies/ml)	p
Before therapy (1)	1175,40±224,23 (95% CI: 787,51 -1666,34)	-
In 1 month (2)	957,92± 178,98 (95% CI: 656,57 -1353,84)	1,2 =0,006
In 2 months (3)	903,72 ± 181,49 (95% CI: 595,53 - 1318,14)	1,3 =0,008; 2,3=0,002
In 3 months (4)	709,72 ± 104,84 (95% CI: 536,11 - 951,70)	1,4=0,00; 2,4=0,00; 3,4=0,00;
After 4 months (5)	715,44 ± 113,68 (95% CI: 530,46 - 958,15)	1,5=0,011; 2,5=0,001; 3,5=0,003 4,5=0,00;
After 5 months (6)	594,64 ± 75,87 (95% CI: 470,27 - 778,07)	1,6=0,001; 2,6=0,001; 3,6=0,001; 4,6=0,001; 5,6=0,001;
After 6 months (7)	547,20 ± 74,88 (95% CI: 429,90 - 721,18)	1,7=0,001; 2,7=0,001; 3,7=0,001; 4,7=0,001; 5,7=0,001; 6,7=0.001;

Valganciclovir treatment demonstrates a positive trend in DNA copy numbers, as shown in the table. However, after 6 months of continuous antiviral therapy, HHV6 DNA copy number remains elevated in saliva samples in all patients.

Interferon-Alpha and -Gamma Production in Patients Before Therapy

The next step was to study interferon-alpha (IFN-α) and -gamma (IFN-γ) levels in patients before therapy (Table 2, Figures 2.3).

Table 2: IFN-α and IFN-γ production in patients before therapy

Indicator		Production level (pg/ml)	Reference values (pg/ml)
IFN-α:	serum	5,18 ± 0,13 (95% CI: 4,97 – 5,48)	0 – 5
	spontaneous	6,84±0,89 (95% CI: 5,38 -8,79)	3 – 30
	induced	296,38 ± 46,96 (95% CI: 212,32 - 392,21)	382 - 845
IFN-γ:	serum	5,01± 0,90 (95% CI: 3,41 – 5.76)	0 – 10
	spontaneous	4,44 ± 0,61 (95% CI: 3,36 – 5,71)	0 – 6
	induced	1621,34±284,82 (95% CI: 1107,74 - 2206,76)	281 - 4335

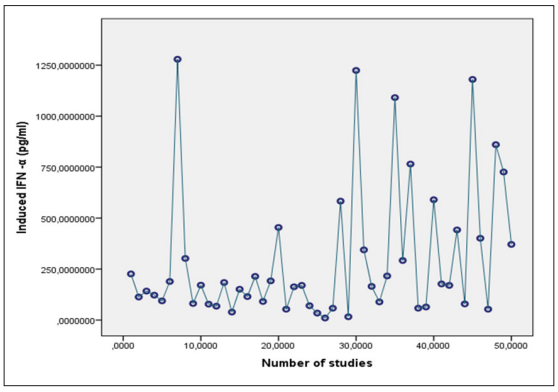

Figure 2: Level of induced IFN-α production in lymphocyte culture.

The table shows that the initial induced IFN-α levels in patients were significantly reduced.

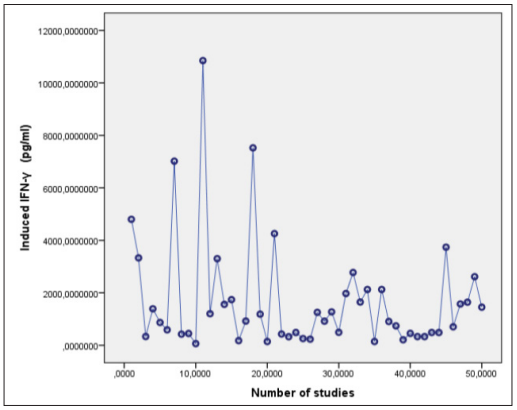

Figure 3: Level of Induced IFN-γ Production in Lymphocyte Culture.

Dynamics of IFN-α and IFN-γ production during valganciclovir therapy

During valganciclovir therapy, the observed changes in IFN-α production in the overall group are presented in Table 3.

Table 3: Dynamics of IFN-α production in patients during valganciclovir therapy

Indicator	Initially	After 3 months	After 6 months	p
	1	2	3	
IFN-α serum	5,18 ± 0.13 (95% CI:4.97 – 5.48)	4.08 ±0.19 (95% CI:3.69 – 4.44)	3.42 ±0.22 (95% CI: 2.95 -3.85)	1,2 =0.001 1,3=0.001 2,3=0.001
IFN-α spontaneous	6.84±0.89 (95% CI: 5.38 -8.79)	5.00±0.40 (95% CI:4.26 – 5.81)	3.84±0.26 (95% CI:3.34 – 4.40)	1,2=0,001 1,3=0.001 2,3=0.001
IFN-α induced	296,38±46.96 (95% CI: 212.32 -392.21)	260.50 ±40.49 (95% CI: 179,37 - 374,74)	249,80 ±41.09 (95% CI: 175,50 -331,93)	1,2=0.001 1,3=0.001 2,3=0.001

The data presented show that after 3 and 6 months of valganciclovir therapy, a significant decrease in IFN-α production was observed, followed by a further reduction in induced production. A similar analysis was conducted in the patient groups with IFN-γ production (Table 4).

Table 4: Dynamics of IFN-γ Production in Patients During Valganciclovir Therapy

Indicator	Initially	After 3 months	After 6 months	p
	1	2	3	
IFN-γ serum	5,01± 0,90 (95% CI:3,41 - 6,90)	3,01 ±0,19 (95% CI: 2,62 -3,41)	2,76 ±0,16 (95% CI: 2,43- 3,09)	1,2 =0.001 1,3=0.001 2,3=0.001
IFN-γ spontaneous	4,44±0.61 (95% CI: 3,36 -5,76)	2,54±0.18 (95% CI:2.18 – 2.92)	2.32±0,16 (95% CI:1.98 – 2.70)	1,2=0,001 1,3=0.001 2,3=0.001
IFN-γ induced	1621,34± 284,82 (95% CI: 1107,74 - 2206,76)	1414,40± 221,18 (95% CI: 1016,88 - 1882,32)	1366,57± 212,84 (95% CI: 938,91 - 1806,88)	1,2=0.001 1,3=0.001 2,3=0.001

The presented data show that after 3 and 6 months of antiviral therapy, a significant decrease in the production of serum, spontaneous and induced IFN-γ is observed, but its values do not go beyond the reference values.

Clinical Complaints in Patients with HHV-6 Infection

Primary HHV-6 infection in adults is rare. Typically, patients experience reactivation of the infection, which leads to the development of clinical complaints, the most characteristic of which are undifferentiated fever or mononucleosis-like syndrome, atypical lymphocytosis, and pruritus. Patients also often develop multiple neurological complaints, weakness, alopecia, fatigue, hearing loss, and gastrointestinal and respiratory symptoms (bronchitis, tracheitis). Patients receive treatment from a neurologist; some consult a psychiatrist, attend psychotherapy sessions, and take antidepressants. However, such therapy does not produce a significant positive effect. Clinical complaints in adult patients with chronic HHV-6 infection have their own specific characteristics [38,39]. We conducted a detailed analysis of the frequency of clinical complaints in these patients before the start of therapy (Table 5).

Table 5: Clinical Complaints in Patients with Chronic HHV-6 Infection before the Start of Therapy

Clinical Complaints	Ratio of patients with complaints to the total number of patients (n=50)
Weakness	46/50
Physical fatigue	42/50
Impaired concentration	40/50
Impaired memory	38/50
Headaches	40/50
Sleep disturbances	39/50
Panic attacks	30/50
Feeling of internal heat	35/48
Unsteady gait	31/50
Unmotivated irritability	46/50
Tearfulness	47/50
Blurred vision	35/50
Increased sensitivity to external sounds and light	27/50
Chills	32/50
Fever	39/50
Sweating	35/50
Myalgia	30/50
Sore throat	23/50
Hair loss	30/50
Lymphadenitis	34/50
Skin rashes	32/50
Pruritus	18/50
Prolonged dry cough	26/50

The table shows that patients present with both neurological and physical complaints, each with a distinctly specific nature. Patients often experience prolonged stress, psycho-emotional, and physical overload, which can lead to a worsening of their clinical complaints.

Changes in Clinical Complaints in Patients Receiving Valganciclovir Therapy after 3 and 6 Months

An analysis of the changes in clinical complaints in patients after 3 and 6 months from the start of valganciclovir therapy is presented in Table 6 and Figure 4.

Table 6: Changes in Clinical Complaints in Patients at Baseline, 3, and 6 Months from the Start of Valganciclovir Therapy

Clinical Complaints	Frequency of complaints at baseline (%)	Frequency of complaints after 3 months (%)	Frequency of complaints after 6 months (%)	p
	1	2	3	
Weakness	92 (46/50)	64 (32/50)	40 (20/50)	1,2=0,001 2,3=0,001 1,3=0.000
Physical fatigue	84 (42/50)	72 (36/50)	36 (18/50)	1,2=0,04 2,3=0,001 1,3=0.000
Impaired concentration	80 (40/50)	62 (31/50)	38 (19/50)	1,2=0,01 2,3=0,001 1,3=0,000
Impaired memory	76 (38/50)	62 (31/50)	36 (18/50)	1,2=0,02 2,3=0,001 1,3=0,000
Headaches	80 (40/50)	66 (33/50)	20 (10/50)	1,2=0,01 2,3=0,001 1,3=0,000
Sleep disturbances	78 (39/50)	60 (30/50)	54 (27/50)	1,2=0,01 2,3=0,01 1,3=0,001
Panic attacks	60 (30/50)	38 (19/50)	20 (10/50)	1,2=0,001 2,3=0,02 1,3=0,000
Feeling of internal heat	70 (35/50)	42 (21/50)	16 (8/50)	1,2=0,001 2,3=0,001 1,3=0,000
Unsteady gait	62 (31/50)	54 (27/50)	28 (14/50)	1,2=0,01 2,3=0,001 1,3=0,000
Unmotivated irritability	92 (46/50)	60 (30/50)	40 (20/50)	1,2=0,001 2,3=0,001 1,3=0,000
Tearfulness	94 (47/50)	60 (30/50)	44 (22/50)	1,2=0,001 2,3=0,001 1,3=0,000
Blurred vision	70 (35/50)	42 (21/50)	22 (11/50)	1,2=0,001 2,3=0,01 1,3=0,000
Increased sensitivity to external sounds and light	54 (27/50)	38 (19/50)	20 (10/50)	1,2=0,001 2,3=0,01 1,3=0,000
Chills	64 (32/50)	40 (20/50)	26 (13//50)	1,2=0,01 2,3=0,01 1,3=0,001
Fever	78 (39/50)	54 (27/50)	30 (15/50)	1,2=0,01 2,3=0,01 1,3=0,001

Sweating	70 (35/50)	54 (27/50)	36 (18/50)	1,2=0,01 2,3=0,02 1,3=0,001
Myalgia	60 (30/50)	50 (25/50)	36 (18/50)	1,2=0,01 2,3=0,01 1,3=0,001
Sore throat	46 (23/50)	34 (17/50)	8 (4/50)	1,2=0,01 2,3=0,000 1,3=0,000
Hair loss	60 (30/50)	42 (21/50)	28 (14/50)	1,2=0,01 2,3=0,01 1,3=0,000
Lymphadenitis	68 (34/50)	40 (20/50)	26 (13/50)	1,2=0,01 2,3=0,01 1,3=0,001
Skin rashes	64 (32/50)	28 (14/50)	20 (10/50)	1,2=0,000 2,3=0,04 1,3=0,000
Pruritus	40 (20/50)	36 (18/50)	28 (14/50)	1,2=0,05 2,3=0,04 1,3=0,02
Prolonged dry cough	30 (15/50)	20 (10/50)	12 (6/50)	1,2=0,01 2,3=0,06 1,3=0,001

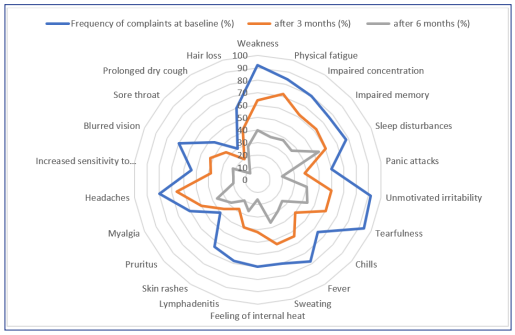

Figure 4: Changes in clinical complaints in patients at baseline, 3, and 6 months from the start of valganciclovir therapy

The data presented in the table show that during therapy and after 6 months of valganciclovir treatment, all patient complaints persisted, with only changes in severity and frequency. None of the patients achieved complete resolution of their symptoms within 6 months of therapy.

Discussion

In 2015, Agut Henri et al. published a study on HHV-6 antiviral therapy, which assessed the choice of antiviral therapy between prophylactic, preemptive, and curative approaches [2]. Therapy can be divided into:

- Preventive therapy was assessed as protecting at-risk individuals from the negative effects of HHV-6 replication or reactivation. In this case, therapy is recommended for all immunocompromised patients, but this carries a risk of developing resistance
- Curative therapy can be recommended after diagnosis of HHV-6 infection. In this case, due to late diagnosis, therapy may be ineffective
- If significant HHV-6 replication is detected, preventive therapy is indicated until the disease itself is diagnosed [2].

The study authors propose the following criteria for initiating therapy.

- confirmed active infection with an elevated viral load in the absence of ciHHV-6;
- immunosuppression;
- concomitant clinical symptoms associated with the pathophysiological replication of the virus;
- absence of other pathogens, in particular human cytomegalovirus.

It is currently accepted that the type of HHV-6, i.e. HHV-6A or HHV-6B, does not play a major role in the decision to initiate therapy, since the relationship between HHV-6 reactivation and diseases has not been sufficiently studied. Transplantologists do not recommend specific therapy, but treatment of the disease itself is based on antiviral therapy with intravenous ganciclovir and/or foscarnet and reduction of immunosuppression. There are currently no definitive recommendations or internationally approved drugs for the treatment of HHV-6 infection. The duration of maintenance therapy to prevent relapse also remains unclear [40,41].

Ganciclovir is known to be the most potent antiviral drug, exerting a pronounced inhibitory effect on viral activity. The drug blocks the synthesis of viral DNA, suppresses the activity of DNA polymerase and reverse transcriptase, which prevents the replication and spread of the virus, has a rapid effect and is long-lasting. Our study demonstrated that valganciclovir exerts a definite antiviral effect against HHV6 reactivation in patients. After six months of continuous valganciclovir therapy, all patients showed a significant decrease in DNA copy number in their saliva samples. However, not a single patient tested negative for PCR in their saliva samples. After three months, a significant decrease in the DNA copy number by 39.62% of the initial HHV6 DNA copy number was observed (from 1175.40±224.23 to 709.72±104.84 copies/ml, p=0.001), and after six months, by 53.45% (from 1175.40±224.23 to

547.20±74.88 copies/ml, p=0.001). In a comparative analysis of therapy in 15 patients who received ganciclovir with a group of 18 patients who did not receive the drug, the authors of the study showed that the average HHV-6 viral load decreased by 0.49 log(10)/week. And in 18 patients who did not receive ganciclovir, the viral load increased by 0.15 log(10)/week (P = 0.04). Thus, the authors concluded that ganciclovir can reduce the HHV-6 viral load in the saliva of patients. However, the presence of DNA copies in the saliva sample in all patients after 6 months of therapy with valganciclovir probably indicates the development of some resistance of HHV-6 to the drug. We assessed the development of resistance only by the absence of a significant positive change in the DNA copy number in the saliva sample, and therefore assumed the development of a viral mutation, the mechanism of which was described in the work of Manichanh, C., et al. The authors of the work showed that the development of resistance to ganciclovir is caused by a viral mutation, which leads to a 24-fold decrease in sensitivity to the drug [30,32,36,42,43].

During the entire therapy period (6 months), patients received a standard daily dose of valganciclovir (900 mg). Previously published study results can be cited that explain the development of acquired viral resistance to valganciclovir with mutations in viral target genes responsible for drug susceptibility in HHV-6: protein kinase and DNA polymerase genes in the case of resistance to ganciclovir and the DNA polymerase gene only in the case of resistance to foscarnet and cidofovir. Interestingly, HHV-6 does not express thymidine kinase, the enzyme that performs the first step of phosphorylation for other antiherpetic drugs such as acyclovir, penciclovir, and brivudine [36,44-46].

Viral infection induces the production of interferons, which bind to interferon-α/β receptors (IFNARs) on the cell membrane. At this point, a transcriptional program involving more than 300 genes that stimulate IFN production is activated, rendering the cell insensitive to viral replication. By producing IFN in response to viral infection, the cell signals other cells to spread the virus. It has been shown that infection of mononuclear cells with HHV-6 leads to an increase in the production of IFN-α; this has also been shown for other herpes viruses. Kikuta, H., et al. studied the production of IFN in peripheral blood mononuclear cells taken from 10 healthy adults seropositive for HHV-6 and five samples of cord blood mononuclear cells after infection with HHV-6. The study's authors demonstrated that 12 hours after exposure of cells to HHV-6, IFN production activity increased, peaking between days 2 and 5 and then gradually declining. IFN production by cord blood mononuclear cells was significantly lower than that of healthy adult cells [47-50].

The interferon-producing cell population consisted of non-T cells and monocytes. The authors concluded that IFN-α production plays a crucial role in the body's response to HHV-6 infection. In contrast, IFN-γ release was suppressed by HHV-6 in peripheral blood mononuclear cells. Our study revealed a decrease in the production of only induced IFN-α and normal levels of serum, spontaneous, and induced IFN-γ. The decrease in the level of IFN-γ production remained within the reference range. These data are inconsistent with the results of the study published by Kikuta, H., et al. This can likely be explained by the fact that the authors of the study assessed IFN-α production after cell infection at 12 hours and after 2 and 5 days. In our study, the duration of HHV-6 infection in patients was over three years. This fact plays a role in the decrease in induced IFN-α production in patients. IFN-γ production values before therapy were within the reference range in our study. This was previously shown in the work of Mayne, M., et al., who demonstrated no change in IFN-γ production in continuous T cells immediately after infection [51,52].

In vitro experiments have shown that HHV-6B is a strong inducer of IFN-α and is capable of stimulating the Th1 response and blocking the Th2 response. During therapy, after 3 months and after 6 months of taking valganciclovir, a significant decrease in the level of IFN-α and IFN-γ production was observed. The decrease in interferon production cannot be explained by the influence of the drug valganciclovir, since the main mechanism of action of valganciclovir is the inhibition of viral DNA replication [53].

An assessment of the dynamics of clinical complaints in patients after 3 and 6 months of therapy with valganciclovir was also conducted. Overall, all patients studied can be considered responsive to valganciclovir therapy, as positive dynamics in total neurological and cognitive functions (e.g., gait unsteadiness, headaches, sleep disturbances, impaired concentration, memory loss, panic attacks, unmotivated irritability, tearfulness, visual impairment, increased sensitivity to external sounds and light) were recorded in 43% of patients, and a decrease in total physical complaints (myalgia, fever, chills, sweating, hair loss, itching, lymphadenitis, etc.) was noted in 28.8%. However, it should be noted that after completing the valganciclovir course, all patients continued to experience some isolated complaints. Nevertheless, the therapy was stopped after six months, as the patients' condition had stabilized by the end of the fifth to the beginning of the sixth month. Seventy-two percent of patients (36/50) rated their condition at the end of therapy as "satisfactory," and 28% (14/50) as "good," without any significant complaints. Patients rated the treatment effect as "positive." As a result, all patients believed they had regained their pre-HHV-6 quality of life.

Conclusion

Currently, there are no approved clinical treatment protocols for HHV-6 worldwide, as there is no drug developed specifically for the treatment of HHV-6. There are only recommendations for the use of antiviral drugs for HHV-6 infection. There is a generally accepted opinion that HHV-6 infection is not treatable, and the antiviral drugs used do not provide positive clinical and laboratory results.

A study of patients with chronic HHV-6 infection showed that valganciclovir significantly reduces DNA copy number in saliva samples and reduces the severity of clinical symptoms. Therapy resulted in a positive trend in symptoms after 6 months of valganciclovir treatment. Further studies of other antiviral agents in the treatment of chronic HHV-6 infection appear promising.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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