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STUDY OF THE EFFECTIVENESS OF IMMUNOTROPIC THERAPY OF LONG-COVID-19 PATIENTS WITH TYPE 6 OF HUMAN HERPES VIRUS REACTIVATION

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Introduction. After the acute form of COVID-19, 10 to 30% of patients tend to develop a post-covid syndrome or long-COVID-19. Research is actively being conducted into the causes of long-term COVID-19, one of which may be a violation of the immune response after SARS-CoV2 enters the body as a superantigen and the reactivation of “latent” viruses, in particular, human herpes virus type 6 (HHV6).

Aim. Study of the clinical and virological effectiveness, tolerability, and safety of the medicinal product inosine pranobex in long COVID-19 patients with HHV6 reactivation.

Materials and methods. Anamnestic, clinical, general laboratory, biochemical, molecular genetic studies, and statistical analysis were performed. The study group consisted of 20 patients with long COVID-19 and HHV6 reactivation, 55.0% women and 45.0% men. The control group consisted of 20 practically healthy people of respective age and sex.

Results. The prevalence of HHV6 reactivation in patients with long COVID-19 amounted to 100.0% regardless of the severity of COVID-19 history. The most frequent complaints of patients were increased fatigue in 100.0%; sleep disturbances, constant fatigue, and increased sweating in 85.0%; impaired mobility, headaches, and loss of smell in 80.0%, and others. In patients with long COVID-19 and HHV6 reactivation, changes in the complete blood count were determined compared to the control group of healthy individuals. After the treatment, the patient's condition and laboratory parameters improved significantly. The clinical effectiveness of treatment with inosine pranobex for 12 weeks generally amounted to 60.1%, and the virological efficacy was 79.4%. Long-term treatment of patients with long COVID-19 and active phase of chronic HHV6 infection with the drug inosine pranobex demonstrated safety and good tolerability.

Conclusions. Treatment of long COVID-19 patients with HHV-6 reactivation with the drug inosine pranobex demonstrated 60.1% clinical and 79.1% virological efficacy, good tolerability, and safety.

Keywords: COVID-19, long COVID-19, human herpes virus type 6, immunomodulatory therapy, inosine pranobex.
Introduction

Despite the end of the active COVID-19 pandemic, research into the characteristics of coronaviruses, their impact on the human immune system, as well as the consequences for the body of both the actual disease of COVID-19 and the intensive treatment carried out in most cases continues [1]. Many questions remain, the answers to which still need to be investigated. One of these issues is a condition in several patients after suffering from COVID-19, which is called post-COVID syndrome or long COVID-19 [2]. This condition is noted in 65 million people worldwide, and their number is constantly growing [3]. According to various data, long COVID-19 is observed in 10 to 30% of patients and at least 10% of cases after severe acute respiratory syndrome caused by SARS-CoV2 [4]. Although there is no accurate statistical data in Ukraine, primarily due to population migration due to the full-scale war. However, according to our observations, the symptoms of long COVID-19 are also widespread and occur even in patients after mild COVID-19.

Research is being actively conducted into the causes of COVID-19. According to Vojdani A. et al. (2023), the causes of long COVID-19 were a violation of the immune response after the entry of SARS-CoV2 as a superantigen into the body and the reactivation of “latent” viruses [5-6].

In general, many reports of reactivated herpes virus infections can contribute to the maintenance of post-covid disorders with the probability of neurological, autoimmune, infectious, allergic, oncological, and mixed syndromes [7].

Therefore, the study of associative relationships between long COVID-19 and reactivated herpes viruses is relevant, and the possibility of correcting the therapeutic tactics for such patients requires further study at the evidentiary level.

The aim of our study was to research the clinical and virological effectiveness, tolerability, and safety of the drug inosine pranobex in patients with long COVID-19 and reactivation of the human herpes virus type 6 (HHV6).

Materials and Methods

The study was conducted in compliance with the principles of the 7th revision of the Declaration of Helsinki on Human Rights (2013), the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine. The consent of the Ethics Commission of Danylo Halytsky Lviv National Medical University was obtained (protocol No. 1/22 from 27.02.22).

The study was conducted based on the Department of Clinical Immunology and Allergology of the Danylo Halytsky Lviv National University from September 2020 to December 2023. NICE criteria were used to verify long COVID-19 (COVID-19 rapid guidelines: managing the long-term effects of COVID-19. NICE guidelines. www.nice.org.uk/guidance/ng188) [8].

Inclusion criteria were adults of both sexes aged 18 to 65 with a history of asymptomatic, mild, moderate, or severe COVID-19 and symptoms lasting more than 12 weeks. COVID-19 was confirmed by PCR detection of RNA from nasal/oropharyngeal swabs and antigen rapid tests.

Exclusion criteria: individuals who have suffered from COVID-19 and do not show any long-term complaints, children, pregnant women, HIV-infected patients, patients with autoimmune, oncological, acute infectious and chronic cardiovascular, pulmonary, neurological, lymphoproliferative diseases, diabetes type 1, and type 2, and any other accompanying decompensated diseases, as well as individuals who abuse alcohol, narcotic substances or were exposed to toxic or chemical substances.

A general examination of patients and basic paraclinical laboratory examinations were carried out: complete blood count (CBC), urine, levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and uric acid. To be included in the study and to confirm the presence of HHV6, molecular genetic analyses were performed to detect HHV6 DNA in three environments (blood, saliva, mucosa).

Patients’ examination data with long-COVID-19 were entered into the author’s “Questionnaire of Clinical and Laboratory Data” improved by us, which is attached.

A total of 111 people (mean age 42±6.7 years old) who suffered from COVID-19 in the period from September 2021 to April 2022, and 20 (18.0%) of them revealed symptoms for more than 12 weeks, who were included in the study group as patients with long COVID-19, of which 11 (55.0%) were women, and 9 (45.0%) were men.

According to the results of the analysis of anamnestic data, before COVID-19, the patients considered themselves practically healthy (which met the inclusion criteria). The regular intake of medicinal drugs was denied, with...
painkillers (non-steroidal anti-inflammatory drugs), vitamins, herbal sedatives, local antiseptic, and disinfectants being periodically taken only when necessary.

Suffered asymptomatic form of COVID-19 – 1 (5.0%) person, mild – 6 (30.0%), medium-severe – 10 (50.0%), severe COVID-19 – 3 (15.0%) persons. 13 people were treated in hospital conditions, 11 of them received extracorporeal oxygen therapy, and in no case was there a connection to COVID-19 requiring artificial pulmonary ventilation device. Antiviral, antibacterial, or antifungal drugs, low-molecular-weight heparins, systemic corticosteroids, etc., were used during the treatment. The average number of days of hospital stay amounted to 11±3.5.

The control group consisted of 20 practically healthy persons of the appropriate age and gender (no confirmed history of COVID-19).

Inosine pranobex 1000 mg tablets were used at a dose of 50 mg/kg/day for 12 weeks to treat patients with long COVID-19 with replicative activity of HHV6.

Statistical analysis

The descriptive statistical method was used to process the empirical data of patients from research groups, their systematization and quantitative description, and visual presentation in the form of figures (graphs). In the case of normal (Gaussian) data distribution, the Student’s t-test was used. In the case of non-normal data distribution, the non-parametric Mann-Whitney U-test was used. To compare data between the study and control groups, the calculation of the arithmetic mean value and its standard deviation (M±SD) was used, with p<0.05 significance of the difference in the results obtained in the groups (probability of error when rejecting the null hypothesis according to the results of the Student’s test). To assess the significance of the difference in the variances of two random samples, Exact Fisher’s test was used for the difference in the proportion of one variable depending on the value of the other variable in the analysis of randomness tables, and the result p<0.05 was considered statistically significant.

Results

All patients were tested for the presence of HHV6 DNA in 3 media. According to PCR data, it was established that in all 100.0% of patients with long-COVID-19, HHV6 DNA was detected in saliva, in 90.0% – in the mucous membrane of the back wall of the pharynx, and in 30.0% – in blood. Thus, the prevalence of HHV6 reactivation in patients with long COVID-19 amounted to 100.0% regardless of the severity of COVID-19 history.

During the clinical examination of long COVID-19 patients with HHV6 reactivation, it was determined that the most frequent complaints of patients were increased fatigue in 20 (100.0%); sleep disturbance, constant fatigue, and increased sweating in 17 (85.0%); impaired mobility, headaches and loss of smell in 16 (80.0%); coughing and impaired memory and attention in 14 (70.0%) patients. Half of the patients (50.0%) had indifference, anxiety, and depressive thoughts, and to a lesser extent, 15.0% to 45.0% complained of chest tightness, loss of taste, hair loss, etc. It should be noted that blood pressure (BP) was measured periodically in patients with headaches, and it was within normal values.

In the full blood count of long COVID-19 patients with HHV6 reactivation, a probable decrease in the levels of segmented nuclear leukocytes (p=0.0001) and an increase in the levels of lymphocytes and monocytes (p=0.0001), as well as ESR (p=0.0001) were determined compared to a control group of healthy individuals.

After the treatment, the patient’s condition generally improved significantly, and most of the clinical manifestations of long COVID-19 decreased significantly: sleep disturbances and loss of smell (p<0.0001), headaches (p=0.0004), constant fatigue (p=0.0003), increased fatigue (p=0.0012), and others. Only a tendency to decreased clinical manifestations of tachycardia (p=0.4801), hair loss (p=0.5145), and chest tightness (p=0.6050) was observed (Fig. 1). Thus, the clinical effectiveness of the treatment of long COVID-19 patients with the replicative activity of HHV6 with inosine pranobex for 12 weeks amounted to 60.1%.
Figure 1. Dynamics of clinical complaints of long COVID-19 patients with replicative activity of HHV6 before and after treatment (n=20)

Improvement of clinical symptoms was accompanied by positive changes in general laboratory indicators. After 12 weeks of treatment, in long COVID-19 patients with HHV6 replicative activity, there was a significant increase in the levels of segmented neutrophils and a decrease in the levels of lymphocytes, monocytes, and ESR compared to both the state before treatment (p=0.0001) and the control group, Table 1.

Table 1

| Dynamics of Complete blood count (CBC) in long COVID-19 patients with replicative activity of HHV6 before and after treatment (n=20) |
|-----------------|-----------------|-----------------|-------|-------|-------|
|                  | Healthy, n=20,  | Long-COVID      | Long-COVID    | P1-2  | P1-3  | P2-3  |
|                  | p1              | +HHV6 before    | +HHV6 after   |       |       |       |
|                  | treatment, p2   | treatment, p3   |                  |       |       |       |
| Leukocytes, g/L  | 6.12±1.21       | 6.29±1.29       | 6.11±1.35      | 0.65  | 1.00  | 0.6850|
| Banded granulocytes, g/L | 2.45±1.43 | 1.70±1.03 | 2.4±1.14 | 0.06  | 0.9033| 0.0486|
| Segmented granulocytes, g/L | 63±5.03 | 40.8±3.49 | 52.7±6.31 | 0.0001| 0.0001| 0.0001|
| Lymphocytes g/L  | 28±4.93         | 43.1±3.29       | 36.35±2.91     | 0.0001| 0.0001| 0.0001|
| Monocytes g/L    | 5.05±1.35       | 12.15±2.39      | 7±1.69         | 0.0001| 0.0003| 0.0001|
| Eosinophils g/L  | 1.5±0.89        | 2.01±1.14       | 2.6±1.96       | 0.123 | 0.0273| 0.2451|
| ESR, mm/h        | 5.9±2.05        | 12.45±3.06      | 6.7±2.23       | 0.0001| 0.2449| 0.0001|
To determine the virological effectiveness of treatment, a comparative analysis of molecular genetic markers was performed in patients with long COVID-19 and HHV6 reactivation who received inosine pranobex before and after treatment.

The analysis of virological efficiency showed that after treatment, the amount of HHV6 DNA in the mucous membrane and saliva significantly decreased (\(p=0.0057, p=0.0001\), respectively), and it was not observed in the blood in any cases (\(p=0.0202\)), Table 2. Therefore, the number of cases of HHV6 DNA in biological media 12 weeks after treatment with inosine pranobex decreased by 3.6 times. The virological effectiveness of treating patients with long COVID-19 against the background of replicative activity of HHV6 with the drug inosine pranobex amounted to 79.4%.

Table 2

<table>
<thead>
<tr>
<th>Medium</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>detected</td>
<td>6 (30%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Saliva</td>
<td>detected</td>
<td>20 (100%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Mucous membrane</td>
<td>detected</td>
<td>18 (90%)</td>
<td>3 (15%)</td>
</tr>
</tbody>
</table>

In case of a pronounced decrease in the clinical manifestations of long COVID-19 and improvement of general laboratory indicators, it can be assumed that 11 (55.0%) patients have entered the latent phase of chronic HHV6 infection.

The safety assessment of the drug inosine pranobex in a daily dose of 50 mg/kg/day was carried out based on a comparative analysis of several biochemical indicators, Table 3.

Table 3

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Healthy</th>
<th>Patients with long-COVID +HHV6</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
<td>1-2</td>
</tr>
<tr>
<td>ALT IU/l</td>
<td>20.1±9.25</td>
<td>21.67±10.19</td>
<td>22.87±11.26</td>
</tr>
<tr>
<td>AST IU/l</td>
<td>18.5±8.64</td>
<td>18.99±8.74</td>
<td>21.07±9.01</td>
</tr>
<tr>
<td>Creatinine mmol/l</td>
<td>72.0±16.67</td>
<td>68.46±17.56</td>
<td>72.39±15.87</td>
</tr>
<tr>
<td>Urine concentration (blood) mmol/l</td>
<td>246.56±56.93</td>
<td>251.68±57.26</td>
<td>274.75±58.74</td>
</tr>
</tbody>
</table>

As evident from the data presented in Table 3, after treatment of patients with long COVID-19 and active phase of chronic HHV6 infection, all indicators of biochemical blood test probably did not change compared to the data before treatment and were within the normal range of healthy individuals of the control group, which demonstrated the safety of long-term use of the drug.

The tolerability of the studied drug inosine pranobex in a daily dose of 50 mg/kg/day in treating patients with long COVID-19 and active phase chronic HHV6 infection was analyzed. According to the assessment of patients, the tolerability of the drug was noted as “good” – 16 (80.0%), and “satisfactory” 4 (20.0%) people. Unsatisfactory tolerability was not determined. In four patients who assessed the tolerability of the drug as “satisfactory,” the following side effects of a mild degree were observed, which did not require the withdrawal of the drug and passed
Independently without the administration of additional treatment: headache – 1 (5.0%) patient, pain in the abdominal cavity – 2 (10.0%), nausea – 1 (5.0%) person. The number of adverse events totaled 4 (20.0%).

Thus, the long-term treatment of patients with long-term COVID-19 and active phase of chronic HHV6 infection with the drug inosine pranobex demonstrated safety and good tolerability.

Discussion

A new syndrome, classified as long COVID-19, is increasingly being detected in a significant percentage of people within a few months after being infected with SARS-CoV-2 [9, 10]. In particular, our study revealed that the prevalence of long COVID-19 amounted to 18.0%. This syndrome is characterized by a wide range of persistent or recurrent or even new symptoms that appear and persist for a long period after an acute infection, regardless of its severity. Meanwhile, patients complain of disorders in various tissues and organs, including respiratory, cardiovascular, gastrointestinal, nervous, and endocrine systems, and disorders of the musculoskeletal system [11-13].

Many studies have shown that the most common development pattern of long COVID-19 is immune dysregulation and a persistent inflammatory process, which is similar to the reactivation of herpesvirus infections [14]. For example, there are some similar symptoms between long COVID-19 and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) as a result of reactivation of latent Epstein-Barr viruses and HHV6 [15]. According to our previous experience and studies, reactivation of HHV-6 was most often detected in patients with long COVID-19. In particular, in this study, the prevalence of HHV-6 in the active phase amounted to 100%. Among the examined patients, the most frequent symptoms were increased fatigue in 20 (100.0%), sleep disturbances, constant fatigue, and increased sweating in 17 (85.0%), also consistent with CFS.

Scientific studies demonstrated that HHV-6 has a broad cellular tropism, the ability to establish a latent period followed by reactivation and persistence in the human body, and several immunomodulatory capabilities [16]. There are reports that HHV-6 can often be detected in tissues affected by autoimmune diseases – it infects cells associated with autoimmune pathology and can alter intracellular signaling and the overall immune response [17-19].

In addition, reactivation of HHV-6 is associated with drug allergy, eosinophilia, and systemic manifestations (DRESS syndrome). According to Zhu H, Ren V. (2023), in particular, reactivation of HHV-6 was detected in 63.0% of patients with DRESS, which was significantly more common than other herpes viruses [20].

Scientists have proposed a new diagnostic strategy for detecting reactivated herpes viruses in patients with long-term COVID-19 to control the formation of autoimmune, allergic, lymphoproliferative, and other pathological processes in these patients [21,22].

Considering the high prevalence of reactivated HHV-6 in patients with long COVID-19 and the previously proven influence of this virus on the immune response with the formation of various disorders, primarily autoimmune, the use of immunomodulatory antiviral therapy in patients with long COVID-19 seems necessary.

Study limitations.

This study had limitations and weaknesses, such as a low sample size and incomplete correspondence regarding the background health status since some patients were temporarily resettled. Our research continues, and determining the immunological effectiveness of inosine pranobex in treating patients with long COVID-19 and reactivated HHV-6 is planned in the future.

In conclusions: Treatment of patients with long COVID-19 and HHV-6 reactivation with the drug inosine pranobex demonstrated 60.1% clinical and 79.1% virological effectiveness, good tolerability and safety. For patients with moderate-severe, severe COVID-19, and long-term COVID-19 symptoms that persist after treatment, therapy with the addition of antiviral-targeted drugs is recommended.

Institutional Review Board Statement: The study was conducted following the 7th revision of the Declaration of Helsinki Human Rights (2013) principles, the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine. Approval was obtained from the Ethics Committee of Danylo Halytsky Liv National Medical University (protocol No. 1/22 dated Feb 27, 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
References


