THE ROLE OF THE HERPESVIRIDAE FAMILY IN THE DEVELOPMENT OF LONG COVID

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Introduction

There is no coherent theory of the causal and pathophysiological mechanisms of the development of long COVID to date. There are many theories, including persistent mild neuroinflammation, mitochondrial damage and dysfunction, autoimmune processes, microvascular dysfunction, SARS-CoV-2 virus persistence, and immune system dysregulation either separately or in combination [1 - 3].

The presence and reactivation of chronic viral infections, such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human immunodeficiency virus, have been suggested as potential factors contributing to the prolonged course of COVID, However, studies in well-characterised cohorts of individuals with COVID-19 over a longer period of time, corresponding to the current definition of long COVID are limited [4].

The aim of this study was to investigate the activity of *Herpesviridae* viruses in leukocytes in patients with long COVID.

Materials and methods

The case-control study included 52 patients with long COVID (Group 1), who were matched with individuals who had COVID-19 and had fully recovered within 3 months of disease onset (Group 2). The patients were examined in the Municipal Multi-Profile Hospital No. 18, Kharkiv City Council, Ukraine.

We used the WHO definition of long COVID, which is a condition usually diagnosed 3 months after the onset of COVID-19, lasting at least 2 months, and not explained by an alternative diagnosis. Symptoms may differ from those observed during the acute episode of COVID-19 or persist from the initial illness [5].

The determination of *Herpesviridae* family antigens was performed by immunofluorescence using specific monoclonal mouse antibodies from MyBiosource, Inc (USA), supplied by Immunogen Sp. zoo (Poland): Mouse Herpes Simplex Virus I, II, Glycoprotein D, Monoclonal Antibody; Mouse HSV-2 gD Monoclonal Antibody; Mouse Varicella Zoster Virus, Glycoprotein B Monoclonal Antibody; Mouse Epstein Barr Virus Monoclonal Antibody; Mouse Cytomegalovirus p65, Monoclonal Antibody; Mouse HHV6 gp60 + gp100, Monoclonal Antibody; FITC labelled Anti Mouse, IgG (H+L), Monoclonal Antibody.

Blood was drawn from the patient's vein. A leukocyte mass was obtained using a standard method. Thin smears of the cell mass were prepared on defatted microscope slides. After drying, the smears were fixed in methanol for 15 minutes, then a working dilution of specific serum was applied to the smears for 15 minutes, and after washing with distilled water, anti-species immunoglobulins labeled with fluorescein isothiocyanate were applied, and the smears were placed in a thermostat at (37 ± 0.5) °C for 25 minutes. After the specified time, the smears were washed with distilled water, dried with filter paper, and examined under a Zeiss Primo Star fluorescence microscope [6].

Statistical data processing was performed based on the variable types. The following were calculated: odds ratio (OR), 95% confidence interval (CI), mode (M), standard deviation (SD), median (Me), ranks, proportions, t-test, $\chi 2$ test.

Results. The Groups were matched for gender (32 women and 20 men in each Group) and age (52.6 ± 21.3 years vs. 50.2 ± 20.6 years, p = 0.56). The mean duration of long COVID in Group 1 was (Me, ranks) - 9.6 [5–13] months.

The activity of *Herpesviridae* viruses in leukocytes is presented in the table.

Table Activity of *Herpesviridae* viruses in leukocytes

Pathogen antigen	Viral load	Group 1 N=52	Group 2 N=52	P
Herpes simplex virus 1,2 (HSV1,2)	Negative result	2 (5.8 %)	9 (17.3 %)	p = 0.026
	Low viral load	8 (15.4 %)	27 (51.9 %)	p = 0.0001
	Medium viral load	29 – (55.8%)	15 (28.9 %)	p = 0.006
	High viral load	13 – (25 %)	1 (1.9 %)	p = 0.0006
Varicella Zoster virus (VZV)	Negative result	14 (26.9 %)	33 (63.5 %)	p = 0.0002
	Low viral load	23 (44.2 %)	15 (28.8 %)	p = 0.15
	Medium viral load	14 (26.9 %)	4 (28.9 %)	p = 0.01
	High viral load	(1.9 %)	0	-

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Pathogen antigen	Viral load	Group 1 N=52	Group 2 N=52	P
EBV	Negative result	21 (40.4 %)	37 (71.1 %)	p = 0.0002
	Low viral load	12 (23.1 %)	13 (25 %)	p = 0.82
	Medium viral load	15 (28.8 %)	2 (3.8 %)	p = 0.0006
	High viral load	1 (7.7 %)	0	-
CMV	Negative result	16 (30.8 %)	34 (65.4 %)	p = 0.0004
	Low viral load	21 (40.4 %)	15 (28.8 %)	p = 0.37
	Medium viral load	21 (40.4 %)	3 (5.8 %)	p = 0.0001
	High viral load	1 (7.7 %)	0	-
Human herpes virus 6 (HHV6)	Negative result	15 (28.8 %)	29 (55.8 %)	p = 0.006
	Low viral load	12 (23.1 %)	15 (32.7 %)	p = 0.28
	Medium viral load	18 (34.6 %)	6 (11.5 %)	p = 0.005
	High viral load	1 (13.5 %)	0	-

As shown in the table, viral load in patients with long COVID was generally higher at medium and high values than at negative or low values. We therefore grouped patients with negative or low values on the one hand, and with medium or high values on the other. Among patients with HSV1/2, 10 (19.2%) had a negative result or low viral load in Group 1 vs 36 (69.2%) in Group 2, $p < 0.0001$. Accordingly, there were 42 (80.8%) patients with medium or high viral load in Group 1 vs. 16 (30.8%) in Group 2, $p < 0.0001$ (Fig. 1).

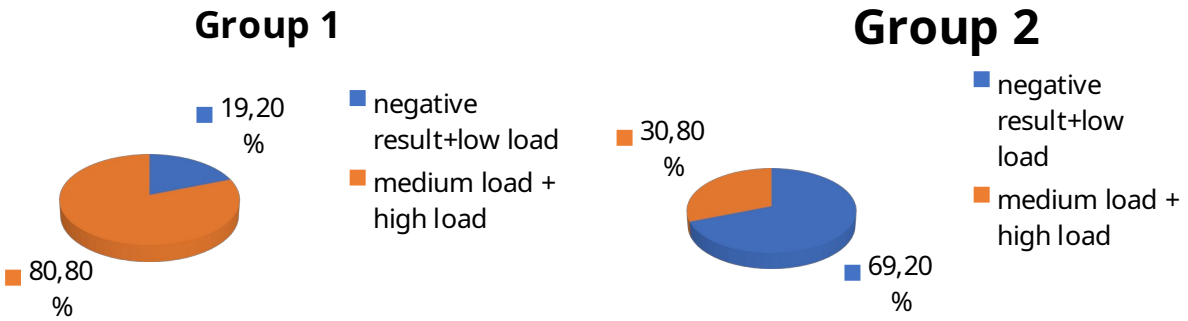

Figure 1 - Viral load HSV 1, 2 in patients with long COVID

The VZV assessment showed similar trends: there were 37 (71.2 %) patients with negative or low load in Group 1 vs 48 (92.3 %) in Group 2, $p = 0.006$. There were 15 (28.8 %) patients with medium and high viral load vs 4 (7.7 %) in Group 2, $p = 0.006$ (Fig. 2).

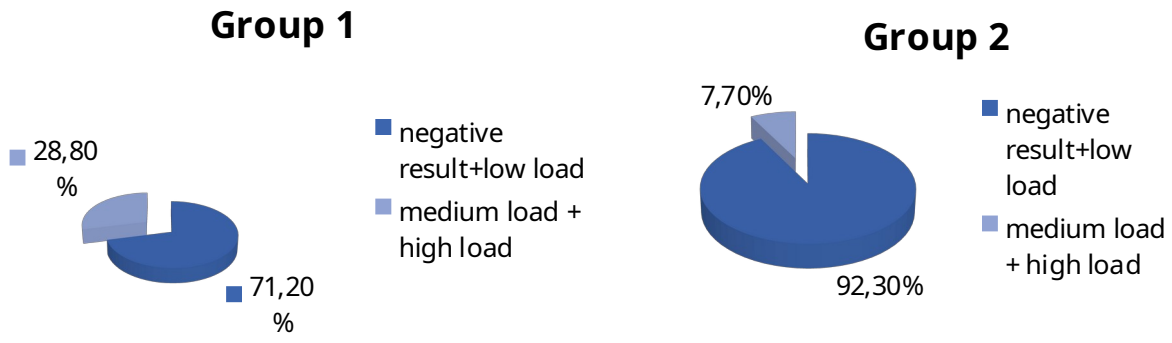

Figure 2 - Viral load VZV in patients with long COVID

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EBV was found in Group 1 in 37 (71.1 %) patients with negative results and low viral load vs 50 (96.2 %) in Group 2, p = 0.0006. There were 15 (28.8 %) patients with medium and high viral load in Group 1 vs 2 (3.8 %) in Group 2, p = 0.0006 (Fig. 3).

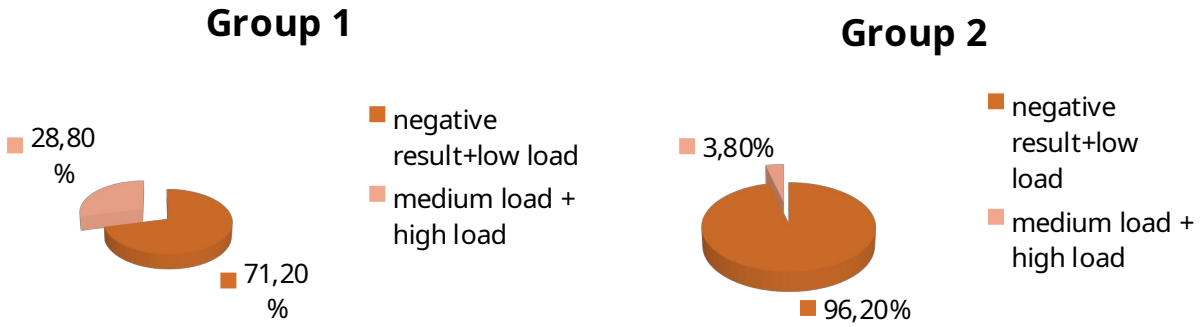

Figure 3 - Viral load EBV in patients with long COVID

CMV: in Group 1 we had 27 (51.9 %) patients with a negative result or low viral load vs 49 (94.2 %) in Group 2, p < 0.0001. Accordingly, there were 25 (48.1%) patients with medium and high viral load in Group 1 vs. 3 (5.8%) in Group 2, p < 0.0001 (Fig. 4).

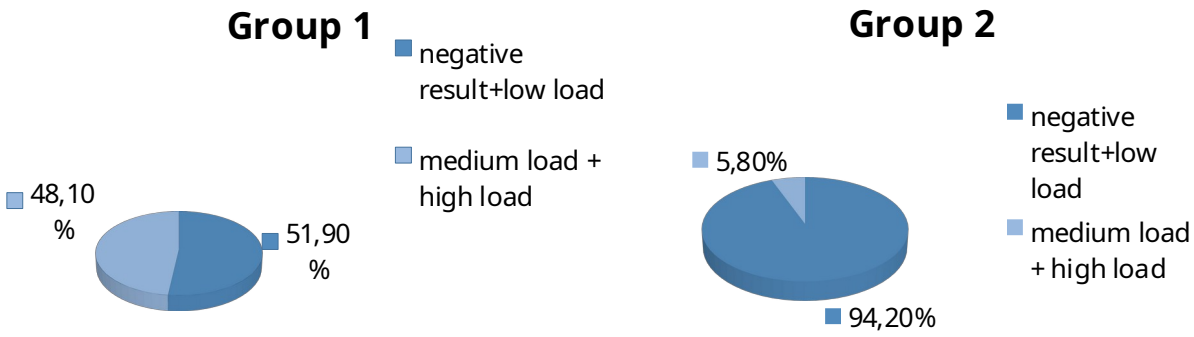

Figure 4 - Viral load CMV in patients with long COVID

Group 1 included 27 (51.9 %) patients with negative results or low HHV6 viral load vs 46 (88.5 %) in Group 2, p < 0.0001. There were 25 (48.1%) patients with medium and high viral load in Group 1 vs 11 (11.5 %) in Group 2, p < 0.0001 (Fig. 5).

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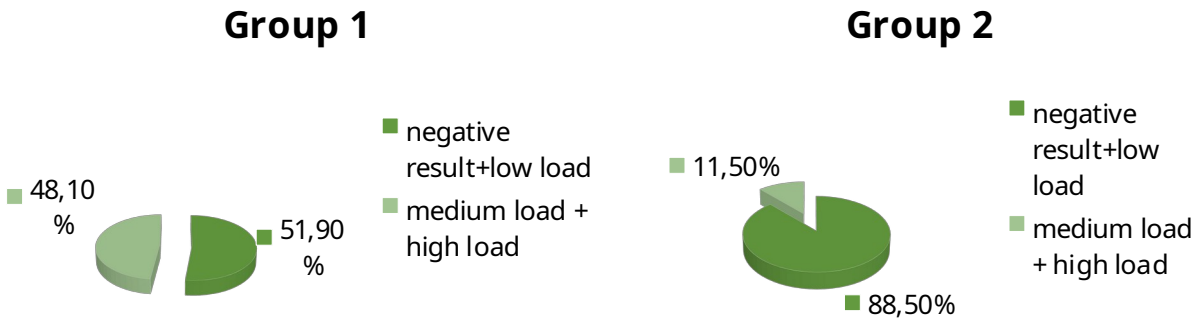

Figure 5 – Viral load HHV6 in patients with long COVID

In general, it can be noted that medium and high HSV1,2 loads were most common: significantly more often than VZV, $p < 0.0001$; EBV, $p < 0.0001$; CMV, $p = 0.0005$; HHV6, $p = 0.0005$. Medium and high VZV loads were equally common as EBV and less common than CMV ($p = 0.004$) and HHV6 ($p = 0.004$). EBV was less common than CMV and HHV6, $p = 0.004$. Finally, CMV and HHV6 were equally common.

Discussion

Overall, we see that EBV and CMV activation are most associated with the development of long COVID. Both EBV and CMV have known neurotropic and endotheliotropic properties, allowing them to infect and persist in the central nervous system and vascular endothelium. Reactivation of these viruses under conditions of immune stress, such as during or after acute SARS-CoV-2 infection, may exacerbate inflammation. New data highlight the interrelated role of herpesvirus infection/reactivation and microvascular endothelial dysfunction. Reactivated EBV and CMV viruses can infect endothelial cells and promote pro-inflammatory states, thereby sustaining a cycle of endothelial dysfunction and neuroinflammation. The interaction between viral reactivation and endothelial damage may contribute to the persistence and severity of long COVID symptoms [7 - 15].

In addition, the persistence and reactivation of latent viruses (e.g., EBV, HSV, HHV6, CMV, and VZV) lead to chronic immune dysregulation, the formation of systemic low-intensity inflammation, and, as a result, multiple organ dysfunction in long COVID-19 [16 - 20].

Several other studies conducted worldwide also confirm the role of Herpesviridae viruses in the development of long COVID. For example, J. E. Gold et al. attempted to establish a link between COVID-19 and viral infections, as well as to link EBV reactivation to long COVID, in a cohort of 185 patients. Of the 185 patients, 56 (30.3 %) reported persistent COVID symptoms. Thirty patients were included in a long-term study (90 days or more after COVID-19 diagnosis), and 20/30 (66.7 %) tested positive for EBV reactivation compared to 2/20 (10 %) in the control Group. Nine patients were included in a short-term study (21 - 90 days after COVID-19 diagnosis), and 79 (77.8 %) tested positive for EBV reactivation compared to 19 (11.1 %) in the control Group [21].

A study by S. Zubchenko and co-authors demonstrated similar results. Eighty-eight patients with COVID-19 were examined, 68 (72.3 %) tested positive for herpesvirus reactivation (EBV, HHV6, CMV) compared to 20 (27.7 %) in the control Group. In patients with symptoms of post-COVID infection, EBV reactivation was observed in 42.6%, HHV6 in 25.0%, and reactivation of both EBV and HHV6 in 32.4%. [22].

We may also encounter other research findings. For example, Ming Yan et al. showed that COVID-19 infection has different effects on the risk of HSV1 and HSV2 infection. infection may reduce the risk of HSV1 infection but increase the risk of HSV2 infection. However, no causal relationship has been found between HSV infection and the severity of COVID-19, the risk of hospitalization, or the overall risk of infection [23].

Conclusions

Our study provides valuable insights into the complex relationship between COVID-19 infection and Herpesviridae infection and offers a scientific basis for developing public health policies and measures. Collectively, it is suggested that latent viral infections should be considered as a causative factor in prolonged COVID-19. Screening and prevention programs may be considered for these conditions.

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Ethics. This study (including informed consent) was approved by the Committee on Biological and Medical Ethics of the SI «I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine» (Protocol No. 1 dated 18.01.2024).

Conflict of interest. The authors declare no conflict of interest.

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The role of Herpesviridae family in the development of long COVID
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Objective. The aim of this study was to investigate the activity of viruses from the family *Herpesviridae* in leukocytes of patients with long COVID. **Materials and Methods.** Following a case-control design, the study included 52 patients with long COVID (Group 1). These patients were matched with "pairs" from those who had contracted COVID-19 and fully recovered within 3 months of disease onset (Group 2). The diagnosis of long COVID was established based on WHO criteria. Detection of Herpesviridae family antigens was performed by immunofluorescence using specific monoclonal mouse antibodies (MyBiosource, USA). **Results.** The Groups were comparable in terms of gender (32 women and 20 men in each Group) and age (52,6 ± 21,3 years vs 50,2 ± 20,6 years), p = 0,56. HSV 1, 2: there were 42 (80,8 %) patients with medium or high viral loads in Group 1 vs 16 (30,8 %) in Group 2, p < 0,0001. VZV: evaluation showed similar trends: 15 (28,8 %) patients had medium or high viral loads in Group 1 vs 4 (7,7 %) in Group 2, p = 0,006. EBV: fifteen (28,8 %) patients in Group 1 had medium or high viral loads vs 2 (3,8 %) in Group 2, p = 0,0006. CMV: twenty-five (48,1 %) patients in Group 1 had medium or high viral loads vs 3 (5,8 %) in Group 2, p < 0,0001. HHV 6: 25 (48,1 %) patients in Group 1 had medium or high viral loads vs 11 (11.5%) in Group 2, p < 0,0001. **Conclusions.** Our study provides valuable insights into the complex relationship between COVID-19 and Herpesviridae infections, offering a scientific basis for developing public health policies and interventions. Collectively, these findings suggest that latent viral infections should be considered a causal factor in long COVID. Screening and prevention programs for these conditions may be considered.
Keywords: Long COVID, *Herpesviridae* family, viral load

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