INTERLEUKIN LEVELS IN THE PATHOGENESIS OF CORONAVIRUS INFECTION (COVID -19) AND THE EFFECT OF A NEW COMBINATION DRUG (G. LUCIDUM AND ALKHADAYA) ON THE COURSE AND PROGNOSIS OF THE DISEASE

Abilov P.M.
*Tashkent Medical Academy (*Tashkent, Uzbekistan)
pulatabilov85@gmail.com

Background. *An outbreak of acute community-acquired atypical pneumonia of unknown etiology was reported in Wuhan, the capital of Hubei Province in central China, in December 2019. The pathogen, which turned out to be a new coronavirus, had not previously been detected in human papillomaviruses and was named SARS-CoV-2. On March 11, 2020, the WHO recognized the NCI epidemic as a pandemic. SARS-CoV-2 belongs to the genus Betacoronavirus family Coronaviridae. This genus also includes the human respiratory pathogens SARS-CoV-1, Middle East respiratory syndrome (MERS) coronavirus MERS -CoV, human coronavirus HCOV-HKU1 and HCOVOC43. Materials and methods of the study. To achieve the set goal, the results of treatment of 200 rats with coronavirus infection caused by COVID -19 were analyzed. The concentration of 27 cytokines was measured: IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1(MCAF), MIP-1a, PDGF- bb, MIP-1b, RANTES, TNF-a, VEGF. Statistical processing was performed using one-way analysis of variance (one-way analysis of variance ANOVA) post Tukey's hoc test or two-way analysis of variance (two-way analysis of variance ANOVA) were used in the work for multiple comparisons between the tested groups. All analyses were performed in a blinded manner with respect to the experimental groups. A P value of <0.05 was considered statistically significant. Results of the research. To determine the level of expression of cytokines and growth factors in coronavirus infection, taking into account different periods of the disease, a multiplex immunoassay was performed. This task was performed with the aim of subsequently identifying protein molecules that have the greatest impact on the modulation of the phenotype in coronavirus infection. The study revealed significant differences in the content of some analytes in coronavirus infection. The level of the proinflammatory cytokine IL-1β was significantly increased on day 3 in all studied groups with coronavirus infection compared to the intact control (P < 0.05). Thus, 95% CI (confidence interval) in III and IV groups is between 2.4-4.0, which indicates an accurate assessment at p≤0.05. The OR (odds ratio) was 0.9523107 between the use of a new drug based on Ganoderma Lucidum and Alkhadaya and the severity of the pathological process in the lungs, χ_2 (Wilconson criterion) is 0.93280714, U (Mann- Whiney criterion) is 0.94135082 at p≤0.05*

Introduction. An outbreak of acute community-acquired atypical pneumonia of unknown etiology was reported in Wuhan, the capital of Hubei Province in central China, in December 2019. The pathogen, which turned out to be a new coronavirus, had not previously been detected in human papillomaviruses and was named SARS-CoV-2. On March 11, 2020, the WHO recognized the NCI epidemic as a pandemic. SARS-CoV-2 belongs to the genus *Betacoronavirus* family *Coronaviridae.* This genus also includes the human respiratory pathogens SARS-CoV-1, Middle East respiratory syndrome (MERS) coronavirus MERS -CoV, human coronavirus HCOV-HKU1 and HCOVOC43 [4]. It is a pleomorphic enveloped virus (size range: 60–140 nm) with characteristic spikes on its surface. The viral genome consists of single-stranded plus-strand RNA [2]. The *ORF1ab sequence,* which makes up two-thirds of the entire genome, encodes a large spike (S) glycoprotein on the virion surface that is essential for binding to the human angiotensin-converting enzyme 2 (ACE2) receptor

and viral entry into the cell [7]. The envelope (E) protein likely forms viroporin, which is important for virus assembly and release, and is also a putative virulence determinant [1]. The membrane protein (M) is an abundantly expressed structural protein within the lipid envelope that is also important for viral morphogenesis and suppression of interferon synthesis [5]. Finally, the nucleocapsid protein (N) stabilizes the RNA genome in the helical complex and serves as a key target of adaptive immunity [8]. In addition, there are a number of accessory proteins, some of which have unknown functions. ORF3a may function as an apoptosis inducer [3]. ORF6 and ORF8 are involved in interferon antagonism, while ORF7a may be involved in inhibition of cellular translation [10]. ORF9b, an accessory protein, is translated from an alternative open reading frame in the gene. The steps of the SARS-CoV-2 replication cycle have been deduced from empirical data and existing knowledge of other β- coronaviruses [9]. The first step of SARSCoV-2 infection is the binding of the S protein to the soluble ACE2 receptor on the host cell surface. ACE2 is known to be expressed on the cell surface of various organs such as the heart, endothelium, liver, kidney, testicles, intestine, lungs and other tissues [6]. Other factors that facilitate viral attachment or entry include neuropilin-1, the UFO receptor tyrosine protein kinase (AXL), CD147, high-density lipoprotein receptor type B1, integrins, angiotensin II receptor type 1 (AT1) and vasopressin receptor subtype 2.

Proteases such as surface TMPRSS2 and endosomal cathepsin L, cleave the S protein, activating viral entry into the cell by endocytosis and membrane fusion. Inside the cell, the virus loses its envelope and the genomic RNA enters the cytoplasm for further replication. Viral assembly occurs in the endoplasmic reticulum, Golgi complex, and intermediate complex (ERGIC), where membranes studded with viral structural proteins interact with the N-encapsulated viral genomic RNA.

Monocytes and macrophages also express ACE2 on their surface and can therefore be infected by SARS-CoV-2, which may lead to the activation and transcription of pro-inflammatory genes. ACE2 receptor expression has been detected on CD68+ and CD169+ macrophages in the lymph nodes and spleen of COVID-19 patients, suggesting that SARS-CoV-2 infects ACE2-positive myeloid cells throughout the body. CD169+ macrophages have been shown to be a site of viral replication, which significantly contributes to the level of viral replication as a result of the state of unresponsiveness to type I IFN-dependent activation, which may contribute to reduced immunity, I proposed a combination of G. Lucidum and Alkhadaya. The composition of this natural product is very wide, including superoxide dismutase, which also reduces the pathogenetic effect of the " cytokine storm" without causing side effects on the liver. I noticed that G. Lucidum and Alkhadaya contain carboxyl groups in their composition, and they are not a continuation of nitro groups and sulfhydryl groups. These carboxyl groups depart from the phenolic rings of G. Lucidum and the benzene rings of Alkhadaya, and the idea of creating a new drug based on G. Lucidum was proposed. and Alkhadaya, and given such great importance not only in the treatment of coronavirus infection, but also in its safe use, then this study is considered a relevant topic and requires further study.

The aim of the study was to determine the value of interleukin levels after the influence of a new combination drug based on G. lucidum and Alkhadaya.

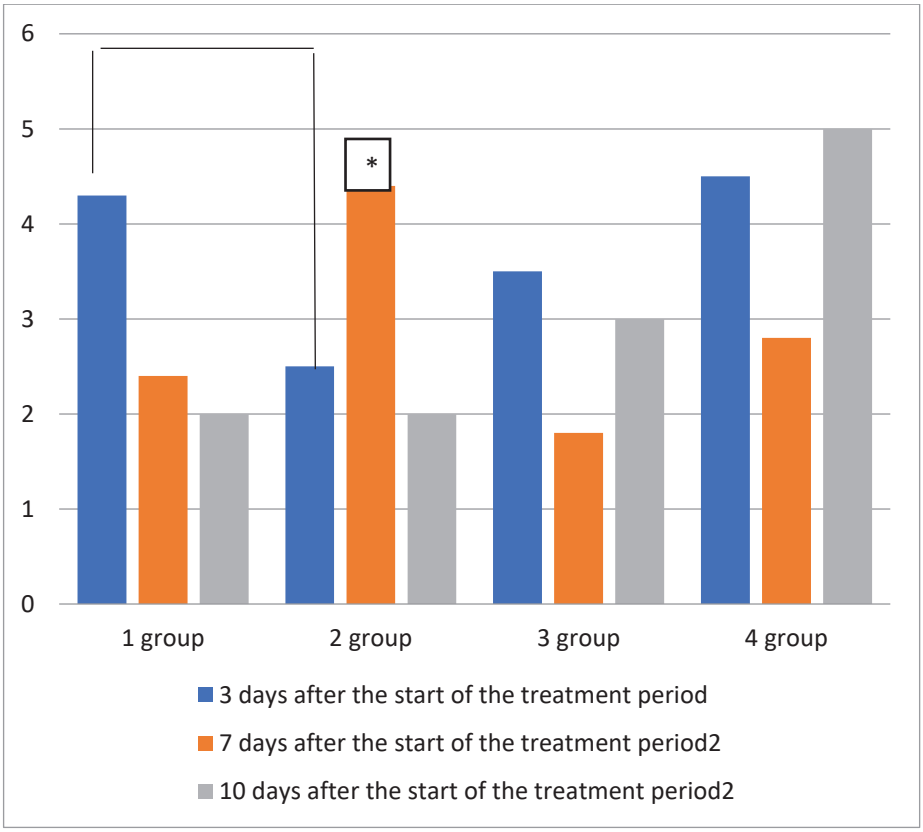
Materials and methods of the study. To achieve the set goal, the results of treatment of 200 rats with coronavirus infection caused by COVID -19 were analyzed. Rats were intranasally infected with the SARS - Cov -2 strain 50% of the tissue culture median infectious dose (TCID 50) per 50 µl of inoculum (biological product with live cultures) after intraperitoneal anesthesia using a 2.5% sodium thiopental solution. All rats were divided into groups: Group 1 - rats with coronavirus infection with a confirmed positive PCR test, treated with ivermectin at a dosage of 300 mg of body weight (n = 50), Group 2 - rats with coronavirus infection treated with baicalin at a dosage of 500 mg (n = 50), Group 3 - rats with coronavirus infection treated with molnupiravir 25 mg / kg of body weight, Group 4 - rats with coronavirus infection treated with a new drug based on G. lucidum and

Alkhadaya. The quantitative profile of cytokines in blood serum was analyzed using Bio-Plex multiplex technology (Bio-Rad, Hercules, CA, USA). The concentration of 27 cytokines was measured: IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1(MCAF), MIP-1a, PDGF- bb, MIP-1b, RANTES, TNF-a, VEGF. Statistical processing was performed using one-way analysis of variance (one-way analysis of variance ANOVA) post Tukey's hoc test or two-way analysis of variance (two-way analysis of variance ANOVA) were used in the work for multiple comparisons between the tested groups. All analyses were performed in a blinded manner with respect to the experimental groups. A P value of <0.05 was considered statistically significant.

Results of the research. To determine the level of expression of cytokines and growth factors in coronavirus infection, taking into account different periods of the disease, a multiplex immunoassay was performed. This task was performed with the aim of subsequently identifying protein molecules that have the greatest impact on the modulation of the phenotype in coronavirus infection. The study revealed significant differences in the content of some analytes in coronavirus infection. The level of the proinflammatory cytokine IL-1β was significantly increased on day 3 in all studied groups with coronavirus infection compared to the intact control ($P < 0.05$).

Fig. 1.

IL-1β level in the study groups in coronavirus infection



**-Mann-Whitney test*

During the same period (day 14), the concentration of IL-4 in group 4 was also minimal, and differences were revealed when compared with group 3. On day 3 after infection, the maximum expression of TNF-α was detected in group 4 when compared with groups 1 and 3. Subsequently, a gradual decrease in the level of TNF-α was observed in this group (4), where on day 14 after infection, the expression of TNF-α was the lowest.

Fig. 2.

IL-4 level in the study groups for coronavirus infection.

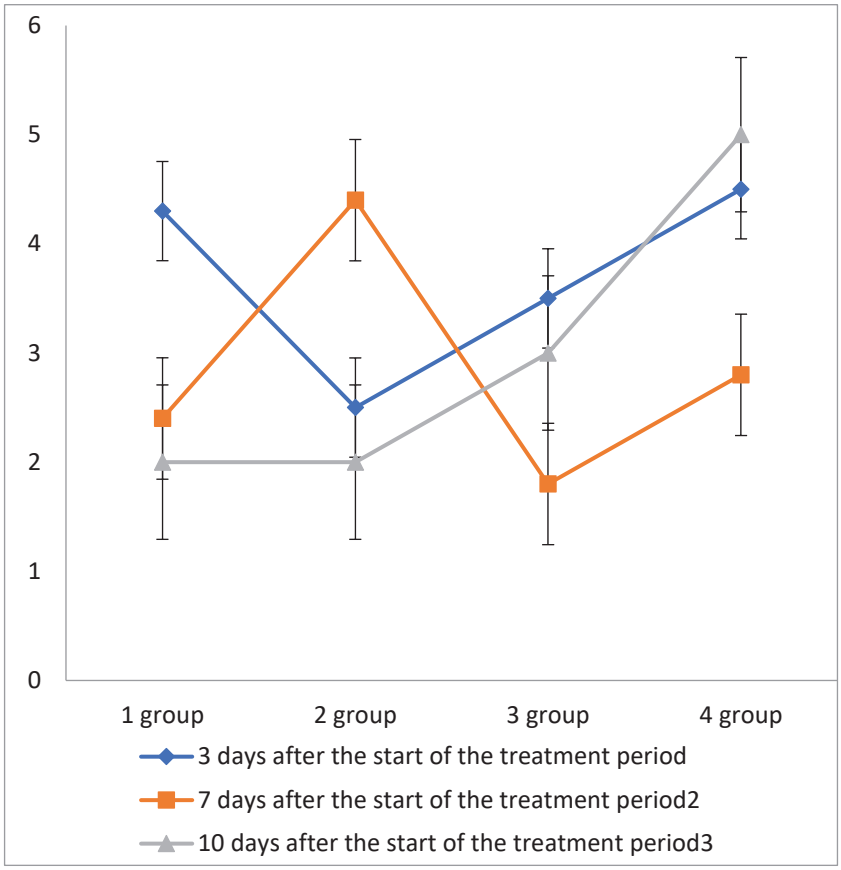
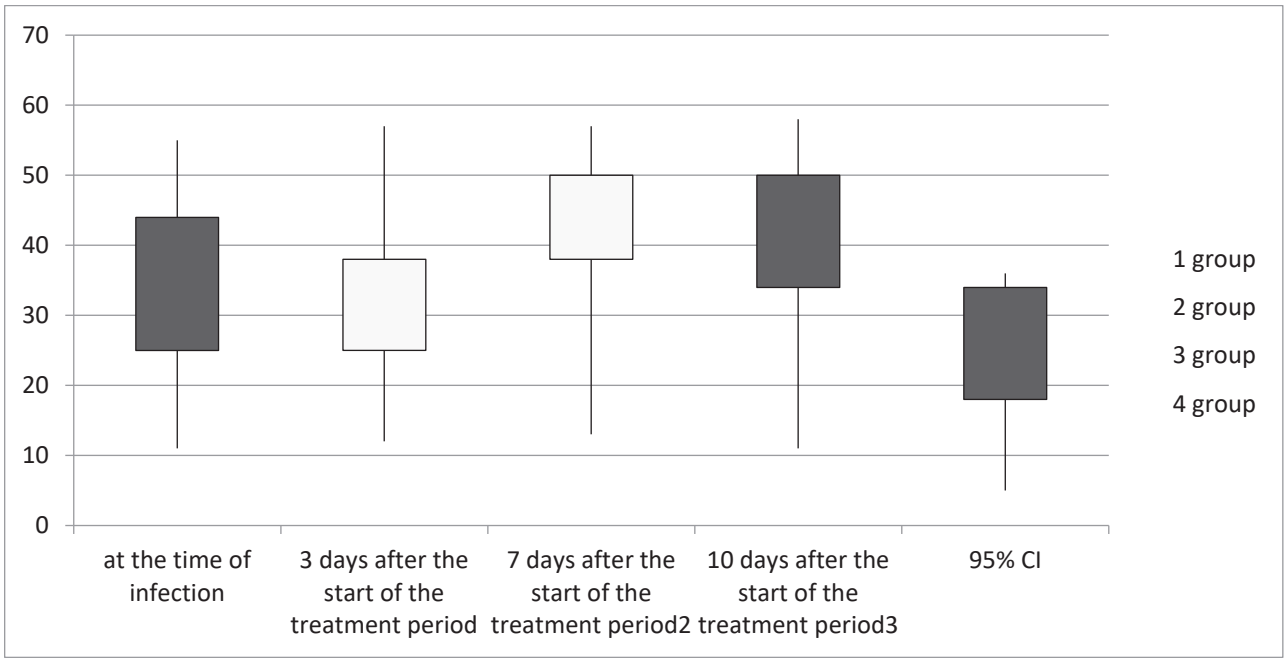


Fig. 3

Correlation relationship between the use of pro- and anti-inflammatory cytokines in coronavirus infection.



CI – confidence interval

Thus, 95% CI (confidence interval) in III and IV groups is between 2.4-4.0, which indicates an accurate assessment at $p \leq 0.05$. The OR (odds ratio) was 0.9523107 between the use of a new drug based on Ganoderma Lucidum and Alkhadaya and the severity of the pathological process in the lungs, χ_2 (Wilconson criterion) is 0.93280714, U (Mann- Whiney criterion) is 0.94135082 at $p \leq 0.05$

Conclusions: The use of cytokines allows predicting the state of spinal cord injury when modeled on laboratory animals.

REFERENCES

1. Ahmed, Hussien et al. A Clinical Review of COVID-19; Pathogenesis, Diagnosis, and Management Bentham Science Publishers https://doi.org/10.2174/1381612826666201222162509

2. Andri Frediansyah et at. Remdesivir and its antiviral activity against COVID-19: A systematic review https://doi.org/10.1016/j.cegh.2020.07.011

3. Clayton B. Hess et at. Immunomodulatory Low-Dose Whole-Lung Radiation for Patients with Coronavirus Disease 2019-Related Pneumonia // International Journal of Radiation Oncology*Biology*Physics Volume 109, Issue 4, 15 March 2021, Pages 867-879

4. European Center for Disease Prevention and Control. Threat assessment brief: emergence of SARS-CoV-2 B.1.617 variants in India and situation in the EU/EEA. May 11, 2021 (https://www.ecdc.europa.eu/en/publications-data/threat-assessment-emergence-sars-cov-2-b1617-variants. opens in new tab).

5. Joint Committee on Vaccination and Immunization. Priority groups for coronavirus (COVID-19) vaccination: advice from the JCVI, 30 December 2020. London: Department of Health and Social Care, January 6, 2021 (https://www.gov.uk/government/publications/priority-groups-for-coronavirus-covid-19-vaccination-advice-from-the-jcvi-30-december-2020. opens in new tab).

6. Matthias Götte et at. Remdesivir for the treatment of Covid-19: the value of biochemical studies Current Opinion in Virology Volume 49, August 2021, Pages 81-85 https://doi.org/10.1016/j.coviro.2021.04.014

7. Ridolo E, Pucciarini F, Barone A, et al. Dermatological manifestations during COVID-19 infection: a case series and discussion on the problem of differential diagnosis. *Acta Biomed.* 2021;92(1):e 2021103. Published 2021 Feb 9. doi:10.23750/ abm.v 92i1.11236

8. Rubin EJ, Baden LR, Udwadia ZF, Morrissey S. India's Covid-19 crisis. N Engl J Med 2021;384(18):e 84-e84.

9. Schreiber G. The Role of Type I Interferons in the Pathogenesis and Treatment of COVID-19. *Front Immunol.* 2020; 11:595739. Published 2020 Sep 30. doi:10.3389/fimmu.2020.595739

10. Zheng, J., Wong, L. R., Li, K. *et al.* COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. *Nature* 589, 603–607 (2021). https://doi.org/10.1038/s41586-020-2943-z