Central Asian Journal of Medicine

PATHOPHYSIOLOGICAL SUBSTANTIATION OF THE USE OF A NEW DRUG BASED ON G. LUCIDUM AND ALKHADIA IN THE TREATMENT OF CORONAVIRUS INFECTION CAUSED BY SARS COV-2.

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ABSTRACT

The current outbreak of coronavirus disease (COVID-19) is a global emergency as its rapid spread and high mortality rate have caused severe disruption. The number of people infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is rapidly increasing worldwide. Patients with COVID-19 may develop pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS) and multiple organ failure.

Key words: coronavirus infection, pathogenesis, acute respiratory syndrome, G. lucidum, Alkhadaya.

INTRODUCTION

The current outbreak of coronavirus disease (COVID-19) is a global emergency as its rapid spread and high mortality rate have caused severe disruption. The number of people infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is rapidly increasing worldwide. Patients with COVID-19 may develop pneumonia, severe symptoms of acute respiratory distress syndrome (ARDS) and multiple organ failure.

Coronavirus is an enveloped positive single-stranded RNA virus. It belongs to the subfamily Orthocoronavirinae, as the name suggests, with characteristic "crown" spines on their surface. Together with SARS-CoV, SARS-CoV of bats and others also fall into the genus of betacoronaviruses [6]. On January 15, 2019 in Taiwan, COVID-19 (caused by 2019-nCoV infection) is classified as a Category 5 Notifiable Infectious Disease. The genus of betacoronaviruses can be divided into several subgroups. 2019-nCoV, SARS-CoV, and bat SARS-like CoV belong to sarbecovirus, while MERS-CoV belongs to merbecovirus, having different biological characteristics and virulence [7, 10].

Immune models of COVID-19 include lymphopenia, lymphocyte activation and dysfunction, granulocyte and monocyte abnormalities, increased cytokine production, and elevated antibodies. Lymphopenia is a key feature in patients with COVID-19, especially in severe cases. CD69, CD38, and CD44 are highly expressed on patients' CD4+ and CD8+ T cells, and virus-specific T cells in severe cases show a central memory phenotype with high levels of IFN-γ, TNF-α, and IL-2. However, lymphocytes exhibit a depletion phenotype with activation of programmed cell death protein-1 (PD1), immunoglobulin T-cell domain and mucin domain-3 (TIM3), and cell lectin-like C-receptor subfamily member 1 (NKG2A) [2, 4]. The level of neutrophils is significantly higher in severe patients, while the percentage of eosinophils, basophils and monocytes is reduced. Increased production of cytokines, especially IL-1β, IL-6 and IL-10, is another key characteristic of severe COVID-19. IgG levels are also elevated and there is a higher titer of all antibodies [8].

Lymphopenia is a key feature in patients with COVID-19, especially in severe cases. Patients with severe COVID-19 are more likely to have lymphopenia on admission, indicating a significant predictor for severe patients. The percentage of lymphocytes has been found to be below 20% in severe cases. Further analysis showed a significant decrease in the number of T cells, especially CD8+ T cells, in severe cases compared to mild cases. Qin et al. reported that the percentage of memory T-helper cells (CD3+CD4+CD45RO+) is also reduced in severe cases compared to non-severe cases. These data indicate that lymphopenia can be used as an indicator of disease severity and prognosis in patients with COVID-19 [3, 5]. However, lymphopenia was present in some non-severe cases and pregnant women; however, the percentage of non-severe patients with lymphopenia is significantly lower than that of severe patients. Interestingly, the number of B cells is within the normal range, which is similar to the results of our study, indicating that damaged B cells are not as significant as damaged T or NK cells [1, 9]. There is also a growing focus on another natural product, the well-known mushroom G.

Lucidum. 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 [10].

Also, in recent years, more and more attention has been paid to another natural product of Alkhadaya, which is black cumin oil. I noticed that both 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 originate from the phenolic rings of G. Lucidum and the benzene rings of Alkhadai, and the idea of creating a new drug based on G. Lucidum and Alkhadai was proposed, and given its importance not only in the treatment of coronavirus infection, but also in its safe use, this study is considered a hot topic and requires further study.

The aim of the study. Optimization of approaches in the diagnosis and treatment of coronavirus infection caused by COVID-19.

Materials and research methods. To achieve this goal, the results of treatment of 50 patients with coronavirus infection caused by COVID-19 were analyzed. All patients were divided into groups: group 1 - patients with coronavirus infection with a confirmed positive PCR test, treated with ivermectin at a dosage of 300 mg of body weight (n=12), group 2 - patients with coronavirus infection treated with baicalin at a dosage of 500 mg (n= 14), group 3 - patients with coronavirus infection treated with molnupiravir 25 mg/kg body weight, group 4 - patients with coronavirus infection treated with a new drug based on G. Lucidum and Alkhadaya.

The amplification reaction and analysis of PCR products were carried out in the mode real time cycler "Rotor-Gene 6000" ("Corbett Research", Australia). The reaction mixtures included oligonucleotide forward and reverse primers complementary to a specific fragment, fluorescent probes labeled with the FAM fluorophore (carboxyfluorescein) and fluorescence quencher (RTQ1), deoxyribonucleoside triphosphates (dNTPs), $MgCl_2$, buffer, Taq polymerase enzyme, and deionized sterile water. For the negative control, the same volume of distilled water was added to the test tube instead of the sample.

Positive samples were determined by the presence of a phase of the logarithmic growth of the fluorescence curve. Registration of results in real time (the value of the threshold cycle, Ct) was performed in tabular and graphical form using computer programs.

Statistical processing was carried out taking into account parametric and nonparametric research methods.

Research results. Taking into account the high analytical sensitivity and specificity of the developed BDags1F/BDags2R primers and the BDags-2P fluorescent probe for the identification of SARS CoV-2, at the next stage of the work, their diagnostic informativeness was studied in the simulation of coronavirus infection in white mice.

In addition, the study used human blood contaminated with SARS CoV-2 cells to evaluate whether primers can be used to diagnose the disease in humans. For this, a suspension of SARS CoV-2 viral cells in yeast form was used with a concentration of 1×10^5 cells/ml to 1×10 cells/ml.

As a result of the work, it was found that the BDags-1F/BDags-2R primers are able to detect virus DNA in the blood at a concentration of 1×10^4 cells/ml. To standardize the experiment in modeling infection, a yeast culture suspension was used at a concentration of 1×10^6 cells/ml. For the amplification reaction, the selection of sectional material from infected white mice was carried out on days 7, 14, 21, and 28 after infection. The study of biological samples using real-time PCR was carried out in parallel with the cultural (mycological) method.

Intraperitoneal infection in susceptible animals may develop a coronavirus infection. The autopsy of animals on the 7th day after infection is due to the fact that this period coincides with the period of manifestation of this coronavirus.

However, the autopsy of infected white mice revealed no macroscopic changes in the internal organs. SARS CoV-2 6/85 DNA was detected by PCR in 12.5% of spleen and blood samples. In the study of sectional material using the cultural method, the growth of SARS CoV-2 6/85 was detected in samples of the liver and spleen in 12.5%.

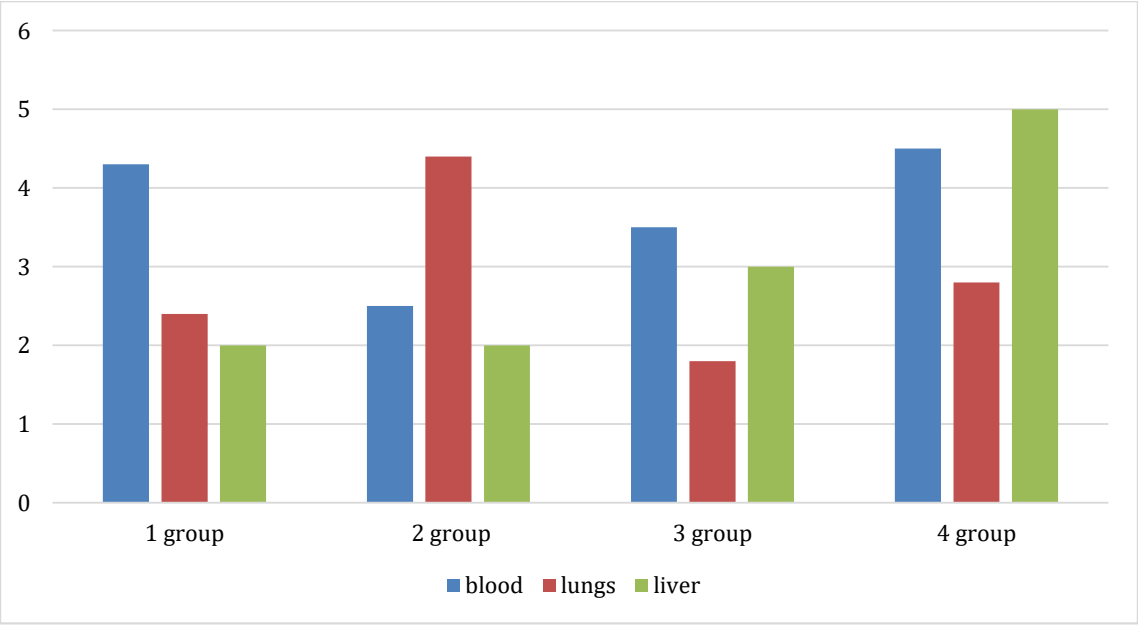

Fig. 1. Results of detection of SARS CoV-2 during experimental infection on the 7th day of the disease.

Thus, the content of T-cells in the norm is 0.8-2.5 109/l. The study revealed a decrease in the content of T-cells ≤0.8X109/l (Fig. 2).

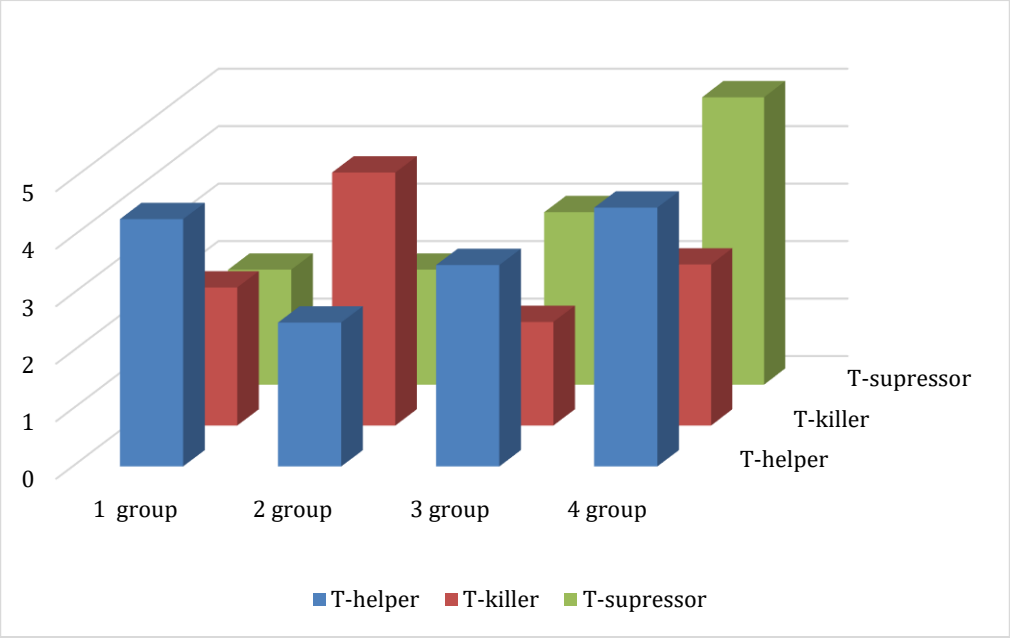

Fig. 2. The content of T-cells at the time of admission of the experimental animal.

Since an increase in the relative number of T-cells is more often observed with a sharply polarized Tx1 type of immune response to a viral antigen, we also observed an increased number of T-lymphocytes.

An increase in the relative number of T-helpers can be observed in the Tx2 type of immune response, which is due to an increase in the number of T-helpers in the Tx2 type of immune response.

Severe Acute Respiratory Syndrome Coronavirus (ARDS) is known to infect cells expressing surface angiotensin converting enzyme 2 (ACE2) and TMPRSS2 receptors, active replication and release of the virus causes the host cell to release damage-associated molecular structures including ATP, nucleic acids and ASC oligomers. They are recognized by neighboring epithelial cells, endothelial cells, and alveolar macrophages, causing the generation of pro-inflammatory cytokines and chemokines (including IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β, and MCP1). These proteins attract monocytes, macrophages, and T cells to the site of infection, promoting further inflammation.

Figure 3 shows the content of B-lymphocytes in coronavirus infection.

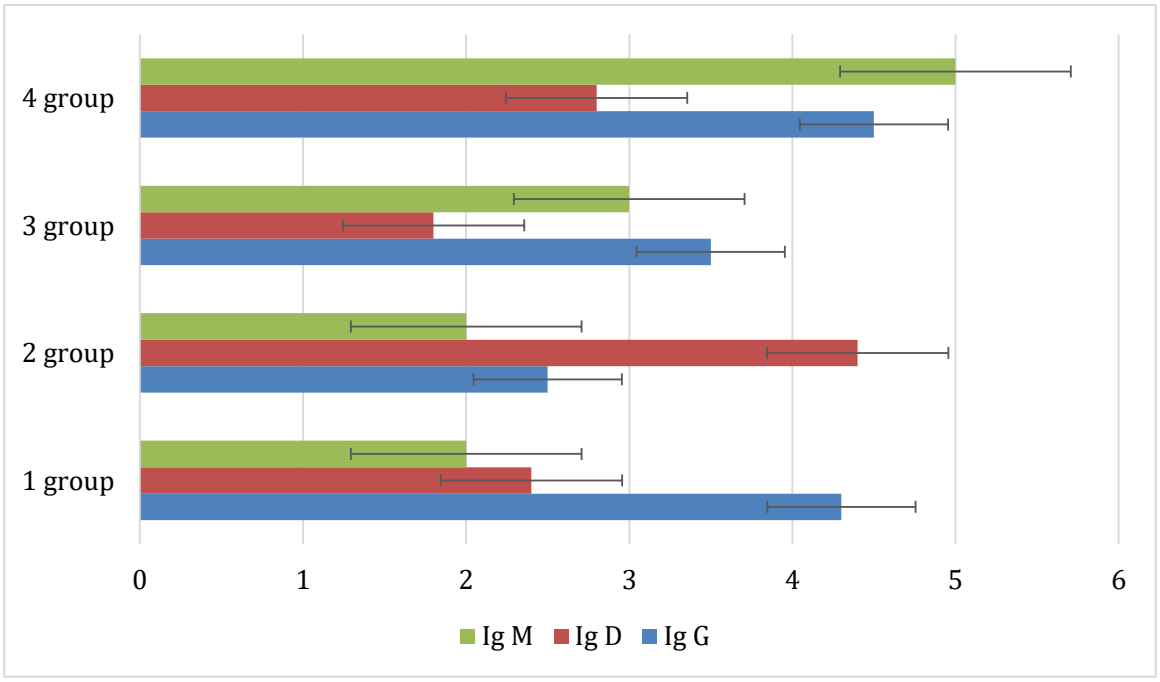

Fig. 3. The content of B-lymphocytes in the peripheral blood of experimental animals with coronavirus infection.

Since a decrease in the absolute number of B-lymphocytes can be the result of lymphopenia, it can be observed in the case of active antibody formation and in coronary infection caused by SARS CoV-2.

After treatment in the 1st study group, on day 14, 10 patients showed an improvement in PCR parameters, which was expressed in a decrease in the degree of detection of SARS CoV-2 in the subjects.

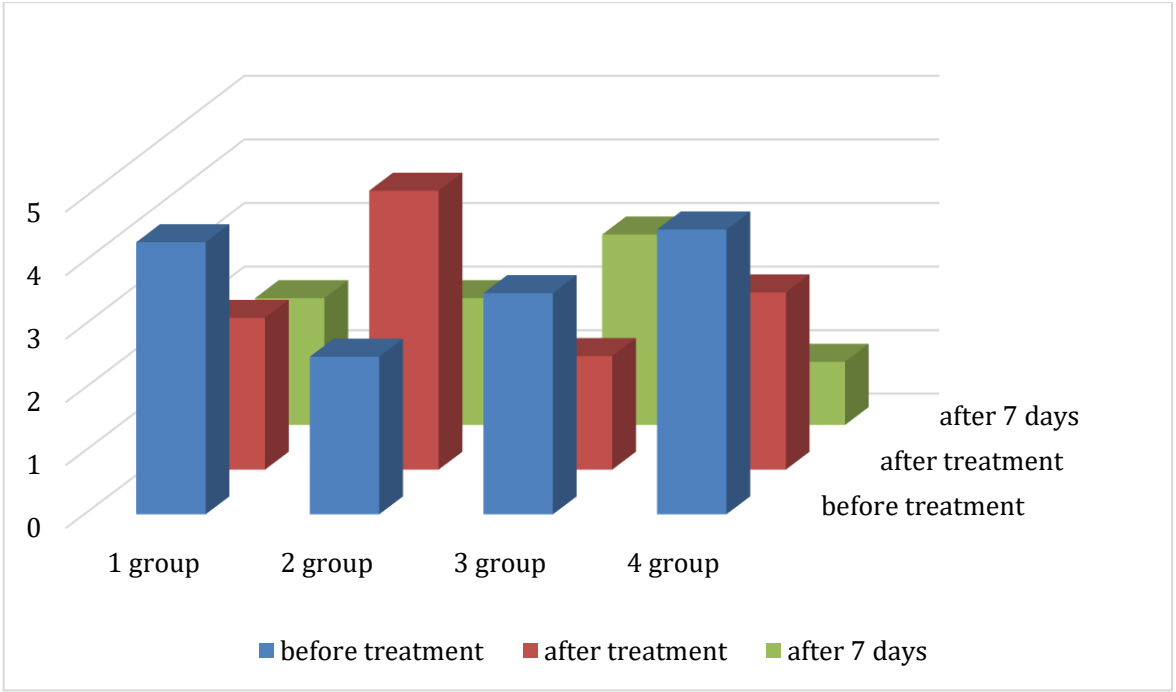

Fig. 4. Results of detection of coronavirus infection caused by SARS CoV-2 in subjects.

Moreover, on day 21, the causative agent of coronavirus infection was found in 25% of liver samples and 37.5% of spleen samples. When examining the abdominal organs of mice at autopsy, the presence of plethora and enlargement of the liver and spleen of infected animals was noted. SARS CoV-2 6/85 DNA was detected by PCR in 12.5% of liver, blood, and 25% of spleen samples.

When modeling an experimental infection, DNA of the causative agent of coronavirus infection by PCR was detected in 10 out of 32 infected mice (31%).

However, the bacteriological method required much more time for research. Visible growth of micromycete was observed only 10-14 days after taking the material for research. Another 7-10 days were required for the appearance of morphological features, with the help of which it was possible to identify the microorganism. Also, the results of PCR were not affected by the possible contamination of samples with foreign microflora.

Table 1.

Comparison of PCR results after treatment in the examined groups.

Groups	PCR results	
1 group	Positive at 6	Negative at 4
2 group	Positive at 9	Negative at 3
3 group	Positive at 8	Negative at 3
4 group	Positive at 1	Negative at 9

Thus, it can be seen that the use of a new drug based on G. Lucidum and Alkhadai is effective in the treatment of coronavirus infection.

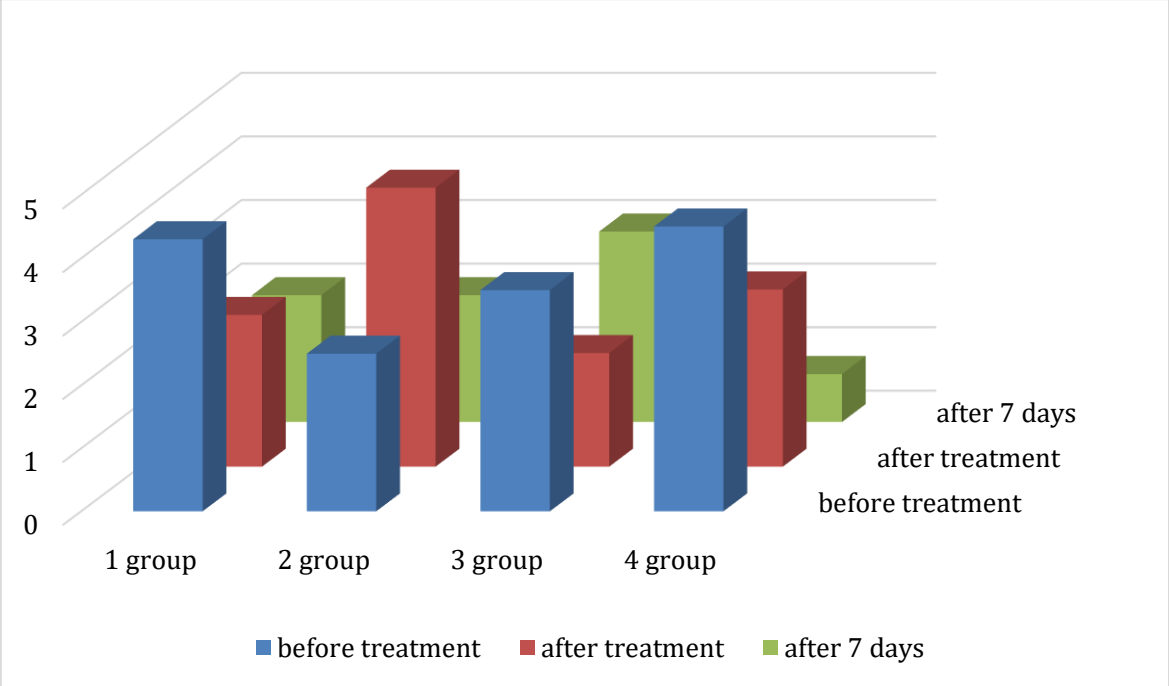

Fig. 5. The content of T-cells after treatment in the examined groups.

There was also an increase in the CD4/CD8 ratio due to an increase in T-helpers (with a normal number of T-killers) and a decrease in CD8 lymphocytes. Since an increase in the CD4 / CD8 ratio due to an increase in CD4 and a decrease in CD8 lymphocytes is usually detected with a mixed Tx1 / Tx2 response to the antigen.

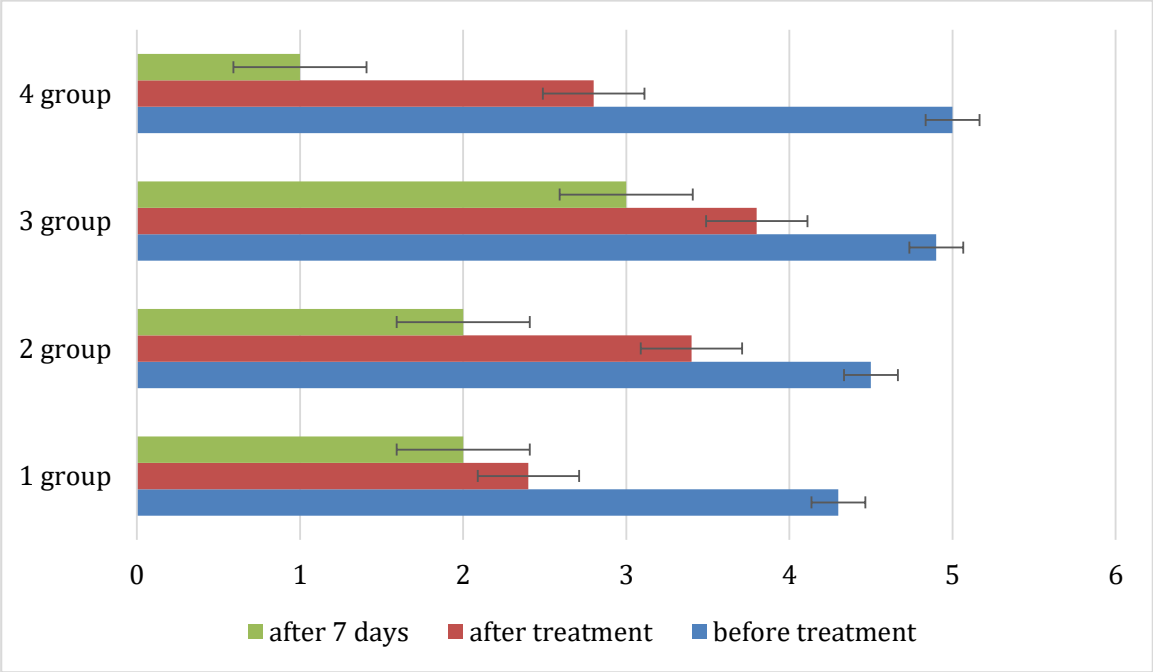

Fig. 6. The content of B-lymphocytes in peripheral blood after treatment.

The results of the presented studies indicate the fundamental possibility of using the polymerase chain reaction to detect SARS CoV-2 DNA. PCR was shown to be more informative in the analysis of blood samples, in comparison with the bacteriological method, which in real conditions can make it possible to detect the pathogen in the early stages of the disease.

Findings. Thus, the detection and elimination of coronavirus infection caused by SARS CoV-2 is achieved after the use of a new drug based on G. Lucidum and Alkhadai (95% CI = 1.3-5.6 at $\chi 2 = 0.9321007$, U (Mann- Winnie) = 0.8721093, N (Kruskes-Wallis test) = 0.9102385 at $p \leq 0.05$.

REFERENCES

1. An experimental assessment of the influence of Ganoderma Lucidum on the state of oxidative stress / Iriskulov B.U., Saydalikhodjaeva O.Z., Abilov P.M., Seytkarimova G.S., Norboeva S.A., Musaev Kh.A. // International journal of scientific & technology research, Volume 9, Issue 03, March 2020: 6645-6649

2. Aromatic constituents from Ganoderma lucidum and their neuroprotective and antiinflammatory activities / Shuang-Yang Li et at. // Fitoterapia, htpps: // doi.org./10.1016/j.fitote.2019.01.013

3. Bioactive metabolites of Ganoderma Lucidum: Factors, mechanism and broad spectrum therapeutic potential / Chetan Sharma et at. // Journal of Herbal Medicine, https: //doi.org/10.1016/j.hermed.2019.10.002

4. Characterization, hypolipidemic and antioxidant activities of degraded polysaccharides from Ganoderma Lucidum / Yu Xu et at. // International Journal of Biological Macromolecules, htpps: //doi.org/10.1016/j.ijbiomac.2019.05.166

5. Clinical and functional evaluation of the effectiveness of treatment of chronic catarrhal gingivitis in children with the use of biologically active additives based on Ganoderma Lucidum // Abilov P.M., Makhkamova F.T. / Pediatric, Scientific and practical journal, №1, 2018: 108-111

6. Comparison on characterization and antioxidant activity of polysaccharides from Ganoderma lucidum by ultrasound and conventional extraction / Qiaozhen Kang et at. // International Journal of Biological Macromolecules, htpps: //doi.org/10.1016/j.ijbiomac.2018.11.215

7. Dayaolingzhiols A-E, AchE inhibitory meroterpenoids from Ganoderma lucidum / Qi Luo et at. // Tetrahedron, htpps: //doi.org/10.1016/j.tet.2019.04.022

8. Development of Ganoderma lucidum spore powder based proteoglycan and its application in hyperglycemic, antitumor and antioxidant function / Li-Fang Zhu et at. // Process Biochemistry, htpps: //doi.org/10.1016/j.procbio.2019.05.025

9. DNA damaging potential of Ganoderma lucidum extracts / Maria Soledad Vela Gurovic et at. // Journal of Ethnopharmacology, htpps://doi.org/10.1016/j.jep.2018.02.005

10. Efficiency of individual prophylaxis of dental caries using dental gel Ispring based on Ganoderma Lucidum in schoolchildren in Tashkent // Abilov P.M. / Journal Dental and Oral Health 5: 1-4, 2018