Original Article

Studying and Improving Treatment of Calprotectin, Helicobacter Pylori and Interleukin-6 in Blood of Patients with Covid-19

M.I. Ismailova[1], A.G. Gadaev[2]

[1]Researcher, Ferghana Medical Institute of Public Health, Ferghana, Uzbekistan.

[2]Researcher, Tashkent Medical Academy, Tashkent, Uzbekistan.

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Abstract

Of the 100 patients who were followed up, 60 were "relatively cured" of Covid-19 and 40 were uninfected. The levels of calprotectin, Helicobacter pylori and interleukin-6 in the blood, which were detected in their feces, were reliably higher in those who passed the coronavirus. At the next stage of the study, the patients of the first group were divided into 2 subgroups of 30 based on the complex treatments prescribed on the basis of the standard of treatment of the existing disease. The first subgroup was prescribed rebamipide as a combination therapy and compared with the other two groups. In the group that received rebamipide, compared to those that did not receive it, it was found that calprotectin, Helicobacter pylori and interleukin-6 in the blood were significantly reduced.

Keywords: COVID-19, Calprotectin, Interleukin-6, Helicobacter Pylori, Rebamipide.

INTRODUCTION

Some researchers put forward the idea that in the first wave of the disease, more respiratory, and in the second wave, symptoms of damage to the gastrointestinal system are characteristic of Covid-19 [9, 13].

In 2019, coronavirus ribonucleic acid (RNA) was first isolated from the feces of a 35-year-old patient who came to the United States with complaints of nausea, vomiting, and diarrhea the next day on the 7th day of the disease [5].

According to further data, the RNA of Covid-19 is detected from the 5th day of the disease, and its peak corresponds to the 11th day. In the feces of some patients, RNA is preserved even after respiratory symptoms disappear and the relevant tests from respiratory organs are positive [16, 18, 12, 17, 2, 15].

It is known that the corona virus enters the body through angiotensin-converting enzyme (AAF2) receptors. Their high expression is observed not only in lung alveolar cells, but also in gastric, duodenal, and rectal glandular epithelial cells [7, 19, 6], and this, in turn, may cause gastrointestinal symptoms in this infection [15].

People with H. Pylori in their body are susceptible to fecal-oral infection. In the observations made in China, people with blood type "A" have been found to be more susceptible to coronavirus than those without this group. In Japan, more than 2,000 people with blood type A were susceptible to H. pylori infection in a randomized controlled trial. These data show that those with blood group A and H. Pylori have a high probability of infecting the human body with the virus through the gastrointestinal system [16., 3, 10, 14].

THE MAIN FINDINGS AND RESULTS

Helicobacter pylori is widespread in Uzbekistan, and 80% of those diagnosed with gastrointestinal diseases have Cag positive strains [1].

It is known that the assessment of inflammatory processes and permeability in the intestine of patients with Covid-19 and its restoration is of great practical importance. Calprotectin can be used to detect inflammatory processes. It is a small calcium-binding protein with a molecular weight of 36 kDa and consists of two heavy and two light polypeptide chains. The protein contains calcium and zinc and has bacteriostatic and fungicidal effects in vitro. Calprotectin is abundant in neutrophils, up to 60% of its cytosol fraction. It is also present in the cytoplasm of monocytes and macrophages. This protein is a product of

neutrophilic granulocytes and its detection in feces indicates the existence of an inflammatory process in the intestinal walls. Since calprotectin protease is considered a stable protein that degrades very slowly under the influence of microorganisms, it is possible to detect it in feces. Therefore, it is a reliable marker of "inflammation".

Restoring inflammatory processes and permeability in intestines of patients with Covid-19 is of great practical importance. Addition of rebamipide to the complex treatment of diseases of the gastrointestinal system in them leads not only to the reduction and disappearance of clinical symptoms, but also to the stabilization of the inflammatory process.

Being a regulator of prostaglandin E2 and I2 synthesis, rebamipid has been proved to have the ability to eliminate the hyperpermeability of the mucous membrane of the gastrointestinal system and is recommended by Maastricht-V as a protector of the gastrointestinal mucosa. It affects the preepithelial, epithelial and subepithelial levels of all parts of the gastrointestinal system. The effect of rebamipid on inflammatory processes is due to the fact that it reduces the activity of neutrophils, reducing their adhesion to epithelial cells and suppressing the production of inflammatory cytokines (interleukin-1, 6, 8, 10, α-tumor necrosis factor). [4, 8]

The reduction of the production of cytokines under the influence of the drug suppresses subclinical inflammatory processes and eliminates the symptoms of the disease and eliminates intestinal permeability.

But in the existing literature, there is no information about the changes observed in "relatively healthy" patients who underwent Covid-19 based on various internal organs, including diseases of the gastrointestinal system. However, their timely detection and secondary prevention procedures are of great practical importance. From this point of view, we set ourselves the goal of studying and improving the treatment of "relatively healthy" patients with symptoms of gastrointestinal system diseases with the help of calprotectin and Helicobacter pylori in feces and interleukin-6 in blood.

Research Material and Methods

Based on the goal, 100 patients with symptoms of diseases of the gastrointestinal system were observed. 42 of them (42%) were men and 58 (58%) were women. They were initially divided into 2 groups. The first group consisted of 27 men and 33 women with an average age of 55.06 ± 2.1, "relatively healthy" but with symptoms of gastrointestinal diseases, who had Covid-19 and did not detect immunoglobulin M.

The second, i.e. control group, was made up of 15 men and 25 women with an average age of 63.4 ± 1.5, who did not have Covid-19 but had symptoms of gastrointestinal diseases.

The first i.e. the main group (those who had Covid-19 and "relatively healthy") were divided into two subgroups based on the treatments carried out. The first subgroup consisted of 30 patients, their average age was 55.2 ± 1.2, 15 were men and 15 were women. The second subgroup consisted of 30 patients, their average age was 56.2 ± 1.2, 12 were men and 18 were women.

In the first subgroup of the main group, rebamipide was administered in addition to standard treatment for their existing disease. Patients took the drug 1 tablet three times a day for 8 weeks. Rebamipide reduces the formation of interleukins by affecting the pathological chain of inflammation. Therefore, the drug is recommended for use as a gastrointerstitial protector by Maastricht-V. Patients in the second subgroup of the main group and in the control group received comprehensive standard treatments according to their existing diseases.

Indicators of calprotectin, Helicobacter pylori and interleukin-6 in the blood of patients under observation were determined:

Calprotectin was assessed in mg by the "sandwich" method (ELISA) using immunoenzyme test RIDASCREEN Calprotectin (R-Biopharm-Germany), and Helicobacter pylori by the immunochromatic test method "RED Helicobacter pylori".

In order to determine interleukin-6 (IL-6) indicators in blood serum, a package of 96 tests was used by the company "AO VEKTOR BEST ROSSIYA". This kit is based on the quantitative determination of the above-mentioned cytokine in human blood serum using an immunoenzymatic assay.

MS Excel (2016) package computer program was used for statistical processing of the obtained data. Arithmetic mean and standard deviation (M±m) of the indicators presented in all tables were calculated. The reliability of differences between groups was determined using Student's criterion for odd and even differences.

Analysis of Research Results

It is of great practical importance to evaluate inflammatory processes and permeability in the intestine of patients with Covid-19 and to restore it. For this purpose, we conducted a series of special biochemical examinations in our patients before the procedures and studied the correlations between them.

It is known that the increase of cytokines in the blood of patients infected with Covid-19 has been proven in a large number of observations. Even in medicine, the term "cytokine flood" has been applied, which causes the death of patients suffering from coronavirus in many cases. However, until now, there is not enough information on the dynamic changes of inflammatory cytokines, in particular interleukin-6, in patients who have clinically recovered from Covid-19. Also, their relationship with

inflammatory markers in the intestinal wall, in particular calprotectin, has not been studied. In our observation, the reliable positive correlation between interleukin-6 and calprotectin confirms that inflammatory cytokines persist for a long time and cause inflammatory processes in intestinal walls even in patients who have undergone clinical recovery after coronavirus infection (Fig. 1).

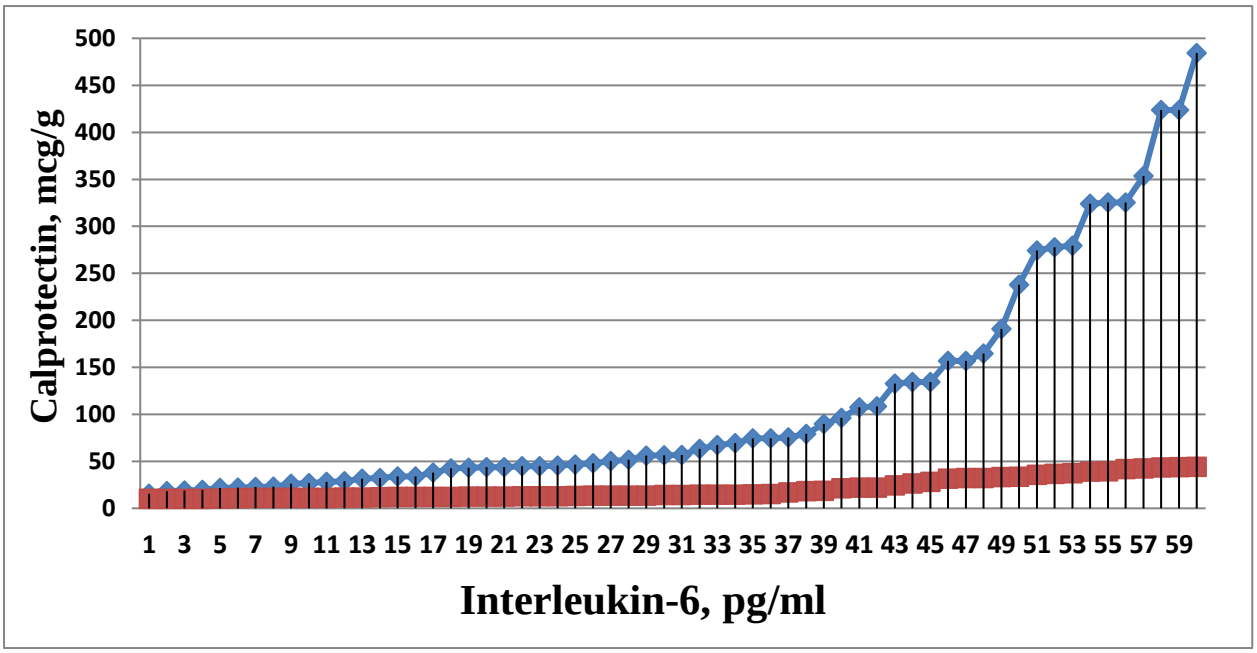

Figure 1. Correlation between interleukin-6 and calprotectin in patients with covid 19 and clinical recovery

Also, the number of patients with Helicobacter pylori detected in the feces of the main (Covid 19) and control (not Covid-19) groups in our observation was compared in percentages and it is presented in Figure 2.

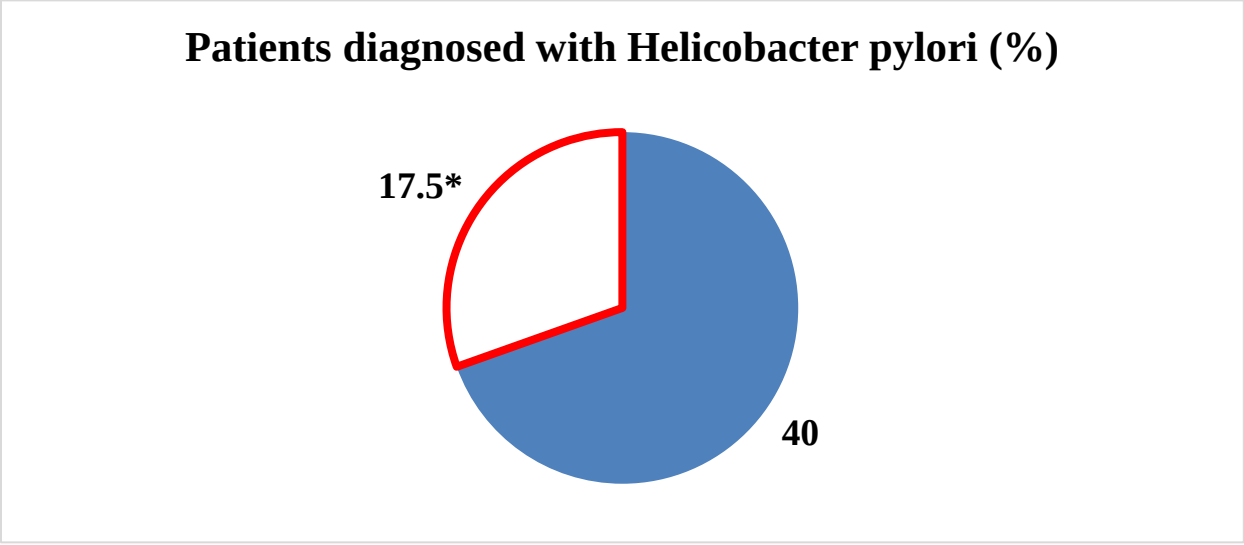

Figure 2. The number of patients diagnosed with Helicobacter pylori in the main (having Covid-19) and control (not having Covid-19) groups in the follow-up (*-R<0.05)

As shown in the picture, H. pylori was detected in the blood of 40% of the main, i.e., "relatively healthy" patients who had Covid-19, and 17.5% of the control (non-Covid-19) patients. When the differences between the two groups were compared, they were reliable (P<0.05). This change can be attributed to the proliferation of H. Pylori in the body as a result of the effects of the coronavirus infection.

Calprotectin values were 114.4±15.88 μg/g and 68.23±12.64 μg/g in the main and control groups, respectively. When the indicators are compared between the two groups reliable (P<0.05) differences were noted (Fig. 3). In patients of the main

group with Helicobacter Pylori detected, calprotectin values were 184, 6±33.25 µg/g, and in those without Helicobacter pylori, its values were 54.8±7.0 µg/g (P<0.0001). In the control group, the indicators were 84.5 ± 29. pg/ml and 57.6 ± 12.5 pg/ml, respectively. The obtained results show that the presence of Helicobacter pylori in the body of the infected with the coronavirus shows that inflammatory processes in the intestines are more evident than those without the infection. High levels of calprotectin confirm the systemic effect of coronavirus infection and the long-term preservation of intestinal inflammatory processes even after clinical recovery.

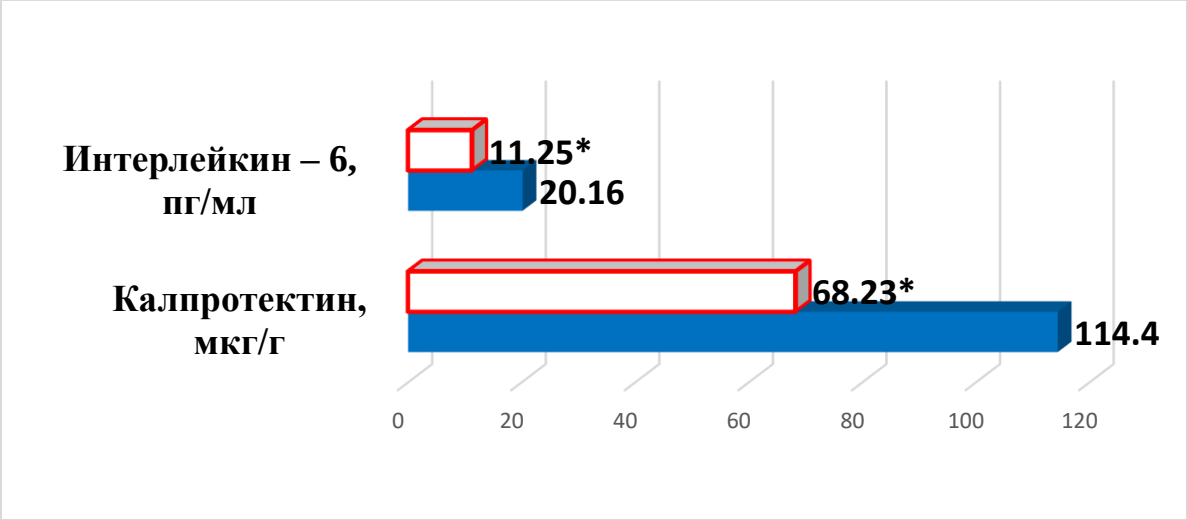

Figure 3. Pre-treatment interleukin-6 and calprotectin levels of patients in the main (having Covid-19) and control (not having Covid-19) groups (*-R<0.05)

In addition to the above, interleukin-6 indicators were on average 20.16±1.44 mmol/l in "relatively healthy" patients who underwent Sovid 19, and 11.25±1.4 mmol/l in control patients, and the differences were highly reliable (P <0.05) (Fig. 3). Interleukin-6 values were 26.1±1.7 µg/g in the main group with Helicobacter Pylori and 14.4±1.74 µg/g in the main group without it, and they were reliably different from each other (P<0.0001). In the control group, the indicators are 14.5 ± 2.49, respectively. pg/ml and 8.0±2.5. was equal to pg/ml. This confirms that inflammatory cytokines remain in dynamics not only during the acute period of the disease, but also after clinical recovery, and indicates that patients need rehabilitation measures.

The coronavirus infection directly affects the epithelia of the mucous membrane of the gastrointestinal tract, causing inflammatory processes. These changes cause an increase in inflammatory cytokines and the development of severe pathological conditions in organs. High levels of inflammatory cytokines and reliable positive correlation with calprotectin in undetectable immunoglobulin M and "relatively healthy" patients with Sovid 19 were also confirmed in our investigations.

In the process of treatment of patients of all groups with Helicobacter pylori detected in feces, treatments consisting of three components (clarithromycin + amoxiclav + ezemeprozole) against this gram-negative bacterium were carried out. Helicobacter pylori was detected in 43.3% and 3.3% of patients before and after treatment, respectively, in the first main group receiving rebamipide based on the standard treatment according to the underlying disease as shown in Figure 4 (R< 0.001). In the second group, which received only standard treatment, Helicobacter pylori was found in 36.6% of patients before treatment and in 26.6% after treatment. In the control group, these indicators were equal to 17.5% and 5% (R < 0.001). The highest effect was observed in the first group of rebamipid drug on the mucous membranes of N. pylori adhesion and reduction of recolonization is associated with increasing the effectiveness of eradication therapy.

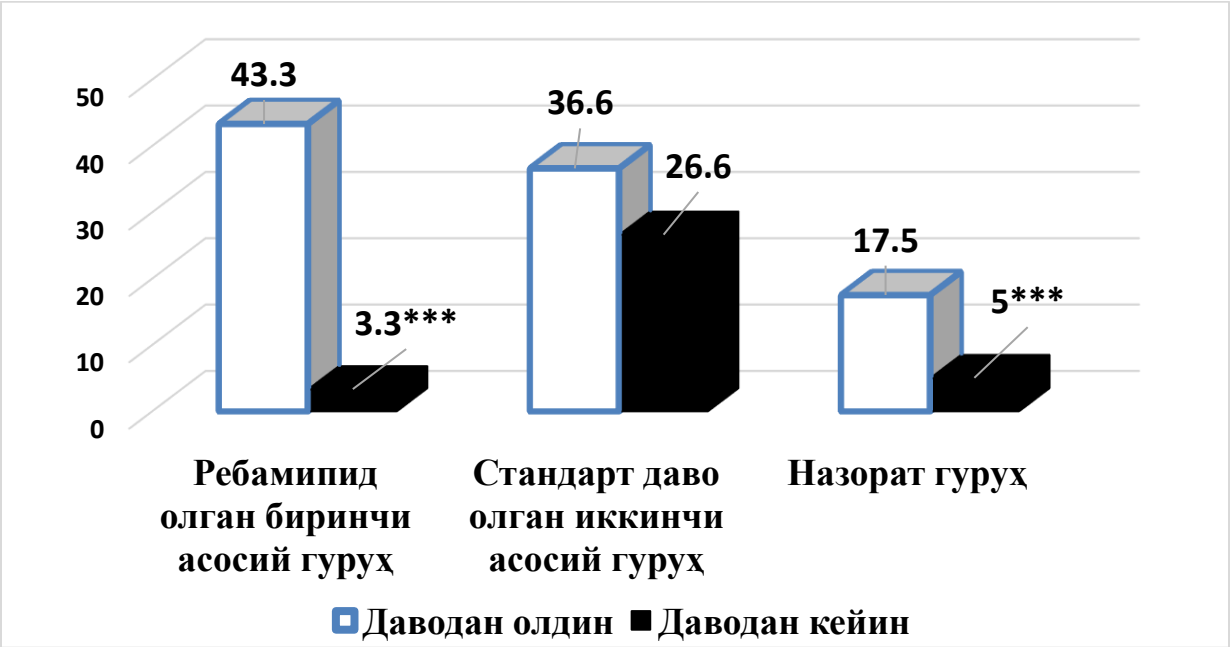

Figure 4. The number of patients with Helicobacter pylori detected before and after treatment in the main (having Covid-19) and control (not having Covid-19) groups in the follow-up (***-R<0.001)

Calprotectin, a marker of intestinal inflammation along with Helicobacter pylori, reliably decreased from 114.4±15.88 µg/g to 48.23±12.64 µg/g in the first group receiving rebamipide with complex treatments (R< 0.01). In the second group, the values before and after treatment were 110.36±12.64 µg/g and 79.01±11.66 µg/g, respectively, and no reliable changes were observed (R>0.05). In the control group, the results obtained before and after treatment (68.23 + 12.6 µg/g and 38.23 + 10.5 µg/g) did not reliably differ from each other (Figure 5).

A reliable reduction of calprotectin in patients of the first group can be attributed to the regenerative effect of rebamipide on mucous membranes.

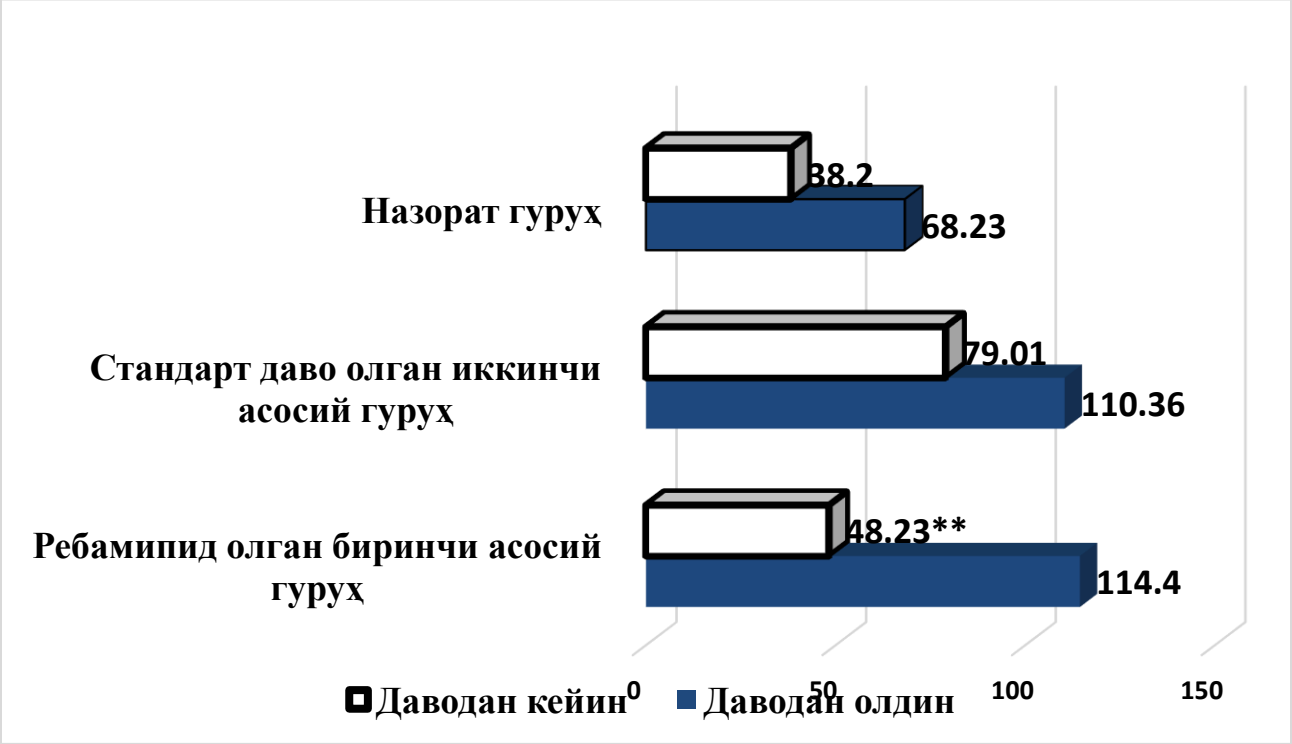

Fig. 5. Pre- and post-treatment calprotectin (µg/g) values of patients in the main (having Covid-19) and control (not having Covid-19) groups (**-R<0.01)

In the first period of our observation, even though high levels of interleukin-6 were detected in all groups of patients, the

numbers were clearly shown in the groups that underwent Covid-19 and their decrease was observed during treatment. Interleukin-6 decreased from 20.16+1.44 pg/ml to 6.25+1.4 pg/ml before and after treatment with high reliability (R< 0.001) in the first group, that is, rebamipide. In the second and third groups, the pre-treatment values decreased from 19.25+1.4 pg/ml to 14.24+0.42 pg/ml (R< 0.05) and 11.25+1.4 pg/ml, respectively. from 6.3+1.4 pg/ml (R< 0.05), they were not highly reliable (Fig. 6).

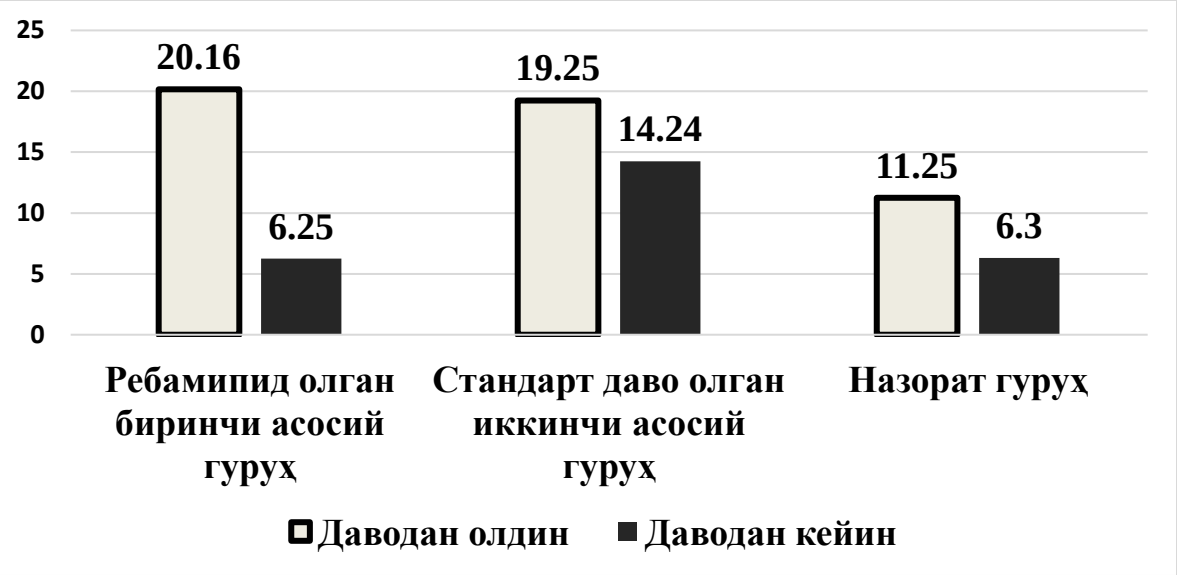

Figure 6. Pre- and post-treatment interleukin-6 (pg/ml) values of patients in the main (Covid-19) and control (non-Covid-19) groups (**-R<0.01)

In the first group, a pronounced decrease in interleukin-6 indicators is associated with rebamipide reducing the activity of neutrophils and the production of inflammatory cytokines in the gastric-intestinal mucosa.

Thus, although positive results were observed after the complex procedures performed in the groups of patients under observation, changes were evident in the group with the addition of rebamipide in all cases. It was observed that the group receiving rebamipide had a reliable decrease in inflammatory processes in the body of the patients, including the gastrointestinal system, and this was shown by the reduction of calprotectin and H.pylori in their feces and interleukin-6 in their blood.

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