



Comparative Analysis of Calprotectin and Helicobacter Pylori in the Faces and Interleukin-6 in the Blood of Patients with and without Covid-19 Before and After the Treatment

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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.

Key words: COVID-19, calprotectin, interleukin-6, Helicobacter pylori, rebamipide.

It has been stated by some researchers, that the first wave of Covid-19 infection affected mainly the respiratory system, and in the second wave, the symptoms of the gastrointestinal system were more characteristic [7, 9].

In 2019, RNA of the coronavirus was isolated for the first time in the USA from the stool of a 35-year-old patient who came with complaints of nausea, vomiting, and diarrhea on the 7th day of the disease [3].

According to a number of studies, 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 the respiratory symptoms disappear and the appropriate tests from the respiratory organs are negative [1, 8, 10, 11, 12, 13].

It is known that the coronavirus enters the body through angiotensin-converting enzyme (ACE 2) receptors. Their high expression is observed not only in alveolar lung cells, but also in gastric, duodenal, and rectal glandular epithelial cells [5, 4] and this in turn may cause gastrointestinal symptoms in this infection [10].

As it is known that the evaluation of inflammatory processes and permeability in the intestines of patients with Covid-19 is of great practical importance. Calprotectin can be used to detect inflammatory processes. Calprotectin is found in neutrophils in abundance and makes up 60 percent 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.

Addition of rebamipide to the complex treatment of patients with diseases of the gastrointestinal system 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 was 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 [2, 6].

Aim of the study: To examine the state of the gastrointestinal organs of patients, recovered from Covid-19 using indicators of calprotectin and Helicobacter pylori in feces and interleukin-6 in blood, as well as improvement of the treatment.

Materials and methods of the study: In accordance with the aim of the study, 100 patients with gastrointestinal symptoms were observed. 42 of them (42%) were men and 58 (58%) were women. Patients were divided into 2 groups. The first group consisted of patients "relatively recovered" from Covid-19 with no detected immunoglobulin M but with gastrointestinal symptoms (27 men and 33 women; average age 55.06 ± 2.1 years).

The second, i.e. control group, consisted of patients, who did not have Covid-19 but had gastrointestinal symptoms (15 men and 25 women; average age 63.4 ± 1.5 years).

The first, i.e. main group (patients who have had and "relatively recovered" from Covid -19) was in turn further divided into two subgroups based on treatment procedures. The first subgroup consisted of 30 patients (15 men and 15 women; average age 55.2 ± 1.2 years). The second subgroup also consisted of 30 patients (12 men and 18 women; average age 56.2 ± 1.2 years).

In the first subgroup of the main group, rebamipide was administered in addition to the standard treatment for their existing disease. Patients took 1 tablet of rebamipide three times a day for 8 weeks. Rebamipide reduces the production 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 treatment suitable to their existing diseases.

The levels of calprotectin and H. pylori in feces as well as interleukin-6 in the blood of patients involved in the study were measured

Analysis of the study 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 treatment procedures and studied the correlations between them.

Also, the presence of Helicobacter pylori in the feces of the main and control groups in our observation were compared. H. Pylori was detected in the feces of 40% of the patients of the main group (i.e. patients who have had and "relatively recovered" from Covid-19), and 17.5% of the control group who haven't had the infection. The differences between the two groups were statistically

reliable ($p < 0.05$). These results can be associated with the increase of *H. Pylori* in the body as a result of the coronavirus infection.

Calprotectin values were $114.4 \pm 15.88 \mu\text{g/g}$ and $68.23 \pm 12.64 \mu\text{g/g}$ in the main and control groups, respectively. Significant ($p < 0.05$) differences were noted when the indicators were compared between the two groups. In patients of the main group, in whom *Helicobacter pylori* was detected, calprotectin values were $184.6 \pm 33.25 \mu\text{g/g}$, and in patients without it, its values were $54.8 \pm 7.0 \mu\text{g/g}$ ($p < 0.0001$). In the control group, the values were $84.5 \pm 29 \text{ pg/ml}$ and $57.6 \pm 12.5 \text{ pg/ml}$, respectively. The obtained results show that the presence of *Helicobacter pylori* in the body of the patients infected with the coronavirus causes more intense inflammatory processes in the intestines than those without the infection. High levels of calprotectin confirm the persistence of inflammatory processes in the intestines even after the systemic effects of the coronavirus infection disappears.

In addition to the above, interleukin-6 indicators were on average $20.16 \pm 1.44 \text{ mmol/l}$ in patients, "relatively recovered" from Covid-19, and $11.25 \pm 1.4 \text{ mmol/l}$ in control patients, and the differences were highly significant ($p < 0.05$). In the patients of the main group, in whom *Helicobacter pylori* was detected, interleukin-6 values were $26.1 \pm 1.7 \mu\text{g/g}$ and in the main group patients without *H. pylori*, the values were $14.4 \pm 1.74 \mu\text{g/g}$ and were reliably different from each other ($p < 0.0001$). In the control group, the values were $14.5 \pm 2.49 \text{ pg/ml}$ and $8.0 \pm 2.5 \text{ pg/ml}$ respectively. This confirms that inflammatory cytokines remain elevated not only during the acute period of the disease, but also after clinical recovery and means 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 its reliable positive correlation with calprotectin in patients "relatively recovered" from Covid-19 with no detectable immunoglobulin M were confirmed in our study as well.

In the course of treatment of patients of all groups, in whose feces *Helicobacter pylori* was detected, treatment consisting of three components (clarithromycin+amoxiclav+ezemeprozole) against this gram-negative bacterium was carried out. Patients of the first subgroup of the main group received rebamipide in addition to the standard treatment for the underlying disease. In these patients *Helicobacter pylori* was detected in 43.3% and 3.3% of patients before and after the treatment, respectively ($p < 0.001$). The second subgroup received only standard treatment. In these patients *Helicobacter pylori* was found in 36.6% and 26.6% of patients before and after the treatment, respectively. In the control group, these values were equal to 17.5% and 5% ($p < 0.001$). The highest effect, observed in the first subgroup, can be explained through effects of rebamipide, which reduces *H. pylori* adhesion to the mucous membranes and their recolonization and increases the effectiveness of eradication therapy.

Calprotectin, a marker of intestinal inflammation along with *Helicobacter pylori*, reliably decreased from $114.4 \pm 15.88 \mu\text{g/g}$ to $48.23 \pm 12.64 \mu\text{g/g}$ in the first subgroup receiving rebamipide in addition to the standard therapy ($p < 0.01$). In the second subgroup, the values before and after treatment were $110.36 \pm 12.64 \mu\text{g/g}$ and $79.01 \pm 11.66 \mu\text{g/g}$, respectively, and no reliable changes were observed ($p > 0.05$). In the control group, the results obtained before and after the treatment ($68.23 \pm 12.6 \mu\text{g/g}$ and $38.23 \pm 10.5 \mu\text{g/g}$) were not significantly different from each other.

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

In the initial period of our observation, even though high levels of interleukin-6 were detected in all groups, the values were clearly higher in patients who have had Covid-19. Their decrease was observed after the treatment. In the first subgroup, namely in patients receiving rebamipide, after

treatment interleukin-6 levels decreased from 20.16+1.44 pg/ml to 6.25+1.4 pg/ml ($p<0.001$). Although in the second subgroup and the control group, there was a decreased in the values from 19.25+1.4 pg/ml to 14.24+0.42 pg/ml ($p<0.05$) and from 11.25+1.4 pg/ml to 6.3+1.4 pg/ml ($p<0.05$), respectively, the changes were less significant.

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

Conclusion. Although positive results were observed after the complex treatment in all groups, in all cases, where patients received rebamipide, changes were more evident. In the group receiving rebamipide a reliable decrease in inflammatory processes in the body of the patients, including the gastrointestinal system was also observed. This was shown by a decrease in calprotectin and H.pylori in their feces and interleukin-6 in their blood.

Resources.

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