



The Effect of Covid-19 on the Blood Supply to the Gums and the Appearance of Caries

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Abstract

The biological system of the oral cavity provides a number of protective mechanisms that fight pathogenic factors that arise due to a decrease in local immunity. This problem is found in patients after Covid-19. There is a violation of the blood supply to all organs and systems, including the mucous membrane of the oral cavity. As a result, patients have an increased risk of ulcers, plaques, fungal infections of the oral cavity, cracks, and spot hemorrhages. Due to a decrease in immune reactions in the oral cavity, the risk of caries in all groups of teeth increases, the permeability of enamel increases, and mineral substances exit from the hard tissues of the tooth.

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1 Introduction

The oral cavity is one of the biological systems of the body that constantly fights pathogenic microflora. Moreover, the oral cavity communicates with both the external environment and the internal one. Therefore, the risk of developing pathological reactions increases due to the

weakening of factors of general, as well as local immunity [1]. Immunoglobulins and lytic enzymes play a special role in immune protection, they are an integral part of immunity, they are alert to pathogens: broad-spectrum bacteria, fungi, and viruses, as well as participate in the regulation and maintenance of an adaptive immune system, the development of inflammatory processes.

One of the most important and main representatives of immunoglobulins in the oral cavity is sIgA, it is an antibody that plays an important role in the immune function of the mucous membranes. The amount of IgA produced in combination with mucous membranes is greater than that of all other types of antibodies combined. Neutrophils are also one of the representatives of the complex mechanism of innate immunity, when a large number of pathogenic or conditionally pathogenic microorganisms appear, they increase the production of cytokines with anti-inflammatory effects [2,3].

2 Literature Review

Nowadays, doctors are faced with a common problem - the coronavirus infection COVID-19. This infection initially affects the respiratory tract, which can be similar both in the mild form, in which patients lose their sense of smell and/or taste, and in severe - with pneumonia, fever and end in death [4,5]. According to the results of recent COVID-19 studies, it was found that patients have dental problems: the appearance of ulcers, plaques, fungal infections, cracks on the lips and tongue, there may also be a change in the color of the mucous membrane, the appearance of hemorrhages, a violation of the integrity of the structure of the hard tissues of the teeth, an increase in regional lymph nodes [6,7]. These problems are the result of a violation of blood supply and the supply of nutrients to the tissues of the teeth and periodontal. Coronavirus infection directly interacts with cellular receptors, thereby it damages the endothelial cells of the vessels of the oral cavity, which in the severe form leads to hypercoagulation and thrombosis, with the combination of these factors, a violation of microcirculation occurs. The second mechanism is associated with inflammatory reactions in the body, it is caused by an increase in the level of cytokines in the blood, in which case the immune cells of the body begin to damage their own tissues (cytokine storm). In addition, the causes of various pathologies of the oral cavity also include medications for the treatment of coronavirus infection [8].

Timely diagnosis of COVID-19 with the involvement of additional microbiological and immunological methods, and observation by a dentist will help prevent the development of undesirable dental symptoms in patients undergoing treatment and rehabilitation. The appointment of symptomatic therapy and preventive measures will help improve the quality of life of a patient with a coronavirus infection [9-11].

3 Method

110 people participated in this study. An immunological study was conducted in 48 patients after coronavirus infection and in 28 people in the control group. Oral fluid was used as a biological material, in which the level of sIgA, elastase and BAPNA-amidase activity was determined [12].

Conclusion: It was found that patients with COVID-19 have an increase in the level of sIgA and BAPNA-amidase activity in the oral fluid, at the same time the level of neutrophil elastase activity is lowered. Statistical values of a decrease in the level of sIgA occur during the rehabilitation period from 3 to 6 months ($p= 0.006$).

According to the activation data, a group of 48 people was formed, from whom oral fluid was taken in order to determine the level of biologically active substances in the oral cavity. The control group consists of 28 people without a history of COVID-19. To assess the state of the biological system of the oral cavity, the relationship between changes in local immunity factors in the oral fluid (the level of sIgA, elastase and BAPNA-amidase activity) and manifestations of coronavirus infection in the oral cavity was revealed. The age of the patients, the presence and degree of damage to the oral mucosa, the presence of carious cavities, the history of medications from the onset of the disease to the end of treatment, as well as during rehabilitation were also analyzed [13,14].

Table 1: sIgA level in oral fluid in patients depending on the rehabilitation period

| N ^o | Comparison Group | The level of mcg/ml, Iu (LQ-UQ) | Reliability |
|----------------|---------------------------|---------------------------------|--|
| 0 | Control (n=28) | 326,94(175,72-551,76) | $p_{0-1}=0,016$; $p_{0-2}=0,013$ $p_{0-3}>0,05$; $p_{0-4}>0,05$ $p_{1-2}>0,05$; $p_{1-3}=0,006$ $p_{1-4}=0,004$; $p_{2-3}=0,028$ $p_{2-4}=0,032$; $p_{3-4}> 0,05$ |
| 1 | Up to 1 month (n=18) | 849(497,6-1403,9) | |
| 2 | From 1 to 3 months (n=12) | 851,9(468,8-1037,9) | |
| 3 | From 3 to 6 months (n=7) | 328(143,8-428,9) | |
| 4 | More than 6 months (n=11) | 230(193,6-372,6) | |

Table 2: The level of sIgA in the oral fluid in patients depending on the duration of the disease

| N ^o | Comparison Group | The level of mcg/ml, Iu (LQ-UQ) | Reliability |
|----------------|-------------------------|---------------------------------|---|
| 1 | 7 days (n=10) | 238, 16 (193,6-428,9) | $p_{1-2}=0,043$; $p_{1-3}=0,038$ $p_{1-4}=0,004$; $p_{2-3}>0,05$ $p_{2-4}=0,019$; $p_{3-4}>0,05$ |
| 2 | 8-14 days (n=9) | 635,27 (421,5-746,2) | |
| 3 | 15-30 days (n=25) | 828,2 (266,47-1206,5) | |
| 4 | More than 30 days (n=4) | 1476,8 (865-2156,1) | |

Table 3: The level of elastase activity in oral fluid in patients with COVID-19

| Comparison Group | Elastase activity level, picocatal, Iu (LQ-UQ) | Reliability |
|--------------------|--|-------------|
| 1. Control (n=28) | 90×10^5 , 20×10^5 - 13×10^5 | $p < 0,001$ |
| 2. Patients (n=48) | $9,1 \times 10^5$, $0,7 \times 10^5$ - $42,3 \times 10^5$ | |

Table 4: The level of BAPNA-amidase activity depending on the duration of the disease

| N ^o | Comparison Group | The level of BAPN-amidase activity mcg/ml, Iu (LQ-UQ) | Reliability |
|----------------|-------------------------|---|---|
| 1 | 7 days (n=10) | 1,64 (1,36-3,09) | $p_{1-2}>0,05$; $p_{1-3}>0,05$ $p_{1-4}=0,024$; $p_{2-3}>0,05$; $p_{2-4}=0,028$; $p_{3-4}>0,05$ |
| 2 | 8-14 days (n=8) | 2,65 (1,42-3,48) | |
| 3 | 15-30 days (n=10) | 3,02 (1,85-4,32) | |
| 4 | More than 30 days (n=4) | 5,78 (4,41-8,93) | |

The study was conducted taking into account patients with a confirmed diagnosis of coronavirus infection by PCR. The study did not involve persons with severe somatic pathology, primary and secondary immunodeficiency, or chronic oral pathology with frequent relapses before COVID-19 disease.



Figure 1: Erythema multiforme localized on the upper and lower lip of the patient after COVID-19.



Figure 2: Vulgar pemphigus on the mucous membrane of the oral cavity of the upper jaw in a patient after suffering COVID-19.

Table 5: The relationship of COVID-19 infection with the incidence of lesions of the oral mucosa

| № | Terms of the disease | Oral mucosa, % | Reliability | Rehabilitation Period | Oral mucosa, % | Reliability |
|---|-------------------------|----------------|---|---------------------------|----------------|--|
| 1 | 7 days (n=10) | 20 | $p_{1-2}=0,08$; $p_{1-3}=0,005$; $p_{1-4}=0,02$; $p_{2-3}=0,16$; $p_{2-4}=0,28$; $p_{3-4}=0,39$ | Up to 1 month (n=18) | 61 | $p_{1-2}<0,001$; $p_{1-3}<0,001$; $p_{1-4}<0,001$; $p_{2-3}=0,5$; $p_{2-4}<0,001$; $p_{3-4}<0,001$ |
| 2 | 8-14 days (n=8) | 55,6 | | From 1 to 3 months (n=12) | 50 | |
| 3 | 15-30 days (n=10) | 56 | | From 3 to 6 months (n=7) | 42 | |
| 4 | More than 30 days (n=4) | 100 | | >6 months (n=11) | 45 | |

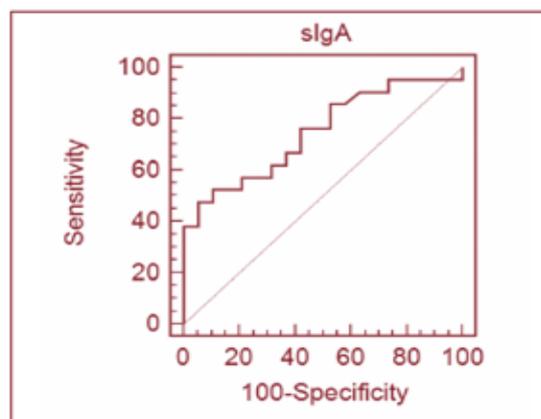


Figure 3: ROC curve of sIgA level in patients after COVID-19

To determine the level of secretory immunoglobulin and enzymes, oral fluid was used, and sampling was performed on an empty stomach. After sampling, the oral fluid was centrifuged in the

laboratory on a centrifuge at 1000 revolutions per minute (200 g) for 10 minutes, the precipitate was collected and stored at a temperature of -70°C until the reaction was set [15]. The content of secretory immunoglobulin (sIgA) in the oral fluid is determined by the ELISA method [16]. To determine the BAPNA-amidase activity of oral fluid, benzoyl arginine-p-nitroanilide was used as a substrate [17]. To determine the activity of elastase, a modified method was used by J. Bailey [7].

4 Result and Discussion

According to the survey, 110 respondents noted the following complaints: loss of smell and taste 63 (57.3%) people, gum inflammation (bleeding, hyperemia, swelling) - 37 (33.6%), a feeling of dryness and burning in the oral cavity-45 (40.9%), the presence of herpetic rashes on the lips and oral mucosa-12 (10.9%) people.

According to the data of the second stage of the study, which involved 48 patients with COVID-19, the average age was 43.7 ± 8.5 ($M \pm \sigma$) years. Groups of patients were organized regarding the duration of the disease: group 1 – 7 days, 10 (20.8%) people; group 2 – 8-14 days, 9 (18.8%); group 3 – 15-30 days, 25 (52.1%); group 4 – more than 30 days, 4 (8.3%) people. Depending on the rehabilitation period, the following were formed:

Group 1 – up to 1 month, 18 (37.5%) people; group 2 – from 1 to 3 months, 12 (25%); group 3 – from 3 to 6 months, 7 (14.6%); group 4 – more than 6 months, 11 (22.9%) people. Manifestations on the oral mucosa were found in 26 examined patients, which amounted to 54.2%, of which 12 (46.15%) people underwent a course of antibacterial therapy.

The median amount of secretory immunoglobulin (sIgA) in the oral fluid in patients after COVID-19 ($n=48$) was 592.67; 254.23– 944.39 mcg/ml (Iu; LQ–UQ), which is higher than in the control group ($n=28$) 326.94; 175.72–551.76 mcg/ml ($p<0.05$). Data analysis showed that there is a negative correlation between the level of sIgA and the rehabilitation period ($r=-50$, $p<0.001$), as well as a positive correlation between the duration of the disease ($r=0.30$, $p<0.001$). No correlation between the sIgA level with the age of patients was found. The analyzed data indicate a prolonged persistence of the virus in the body, the level of sIgA in the oral fluid increases, and even after the end of treatment, the level of immunoglobulin remains high, but a longer rehabilitation period leads to a gradual decrease in patients' indicators to the values of the control group.

When analyzing the level of sIgA in the oral cavity in patients, depending on the duration of rehabilitation, it was found that the decrease in indicators occurs in the range from 3 to 6 months ($p_{1-3}=0.006$; $p_{2-3}=0.028$) and more than 6 months ($p_{1-4}=0.004$; $p_{2-4}=0.032$), values The results obtained during this period did not differ from those of the control group ($p_{0-3}>0.05$; $p_{0-4}>0.05$). In the period up to 1 month ($p_{0-1}=0.016$), as well as from 1 to 3 months ($p_{0-2}=0.013$), the sIgA level remained at a high level in comparison with the control group (Table 1). Depending on the timing of the disease in patients with COVID-19 (Table. 2) there was an increase in secretory IgA after seven days ($p_{1-2}=0.043$; $p_{1-3}=0.038$; $p_{1-4}=0.004$) and increased until the 30th day from the start of treatment ($p_{2-3}>0.05$; $p_{2-4}=0.019$; $p_{3-4}>0.05$). An increase in the level of sIgA in the oral fluid may be due to the manifestation of a "cytokine storm". Active replication and release of the virus trigger

a cascade of reactions in the body, which leads to the development of inflammation. The mechanism of the inflammation process is enhanced by a large number of pro-inflammatory cytokines, for example, IL-6, IL-1, chemokines (IL-8), etc. IL-6 has a pleiotropic effect on acquired immunity, stimulating the production of antibodies by B cells, including sIgA. Normally, inflammation is regulated by anti-inflammatory cytokines, a violation of the regulatory balance leads to a significant destruction of own tissues [6].

Determination of the level of lytic enzymes is important as markers of systemic activation of neutrophils when monitoring the course of infectious and inflammatory processes [10]. The level of neutrophil elastase activity in the oral fluid was analyzed, which was lower in patients with COVID-19 (Table. 3) than that in the control group ($p < 0.001$). When studying this indicator, no changes were detected in patients depending on the duration of the disease and the period of rehabilitation ($p > 0.05$).

In the course of the study, a direct correlation of average strength was established between the level of BAPNA-amidase activity and sIgA ($r = 0.58$, $p < 0.001$).

No correlations were found between the level of sIgA and elastase activity, as well as elastase and BAPNA-amidase activity. BAPNA-amidase activity in patients had a positive correlation of average strength with the duration of the disease ($r = 0.36$; $p < 0.05$), no correlation was found with other criteria. The level of BAPNA-amidase activity did not differ from the control group ($p > 0.05$). In the control group ($n = 28$), the median of this indicator was 2.6; 1.59–3.79 pcat (Iu; LQ–UQ), in patients with COVID-19 – 2.9; 1.46–3.99 pcat (Table 4). When analyzing this indicator in dynamics, it was found that BAPNA-amidase activity increases in patients whose disease duration was from 30 days or more ($p_{1-4} = 0.024$; $p_{2-4} = 0.028$). An increase in the level of BAPNA-amidase activity may be associated with the addition of a secondary bacterial infection against the background of COVID-19.

During the study period, patients after COVID-19 were diagnosed with lesions of the oral mucosa: loss of taste, inflammation of the gums, a feeling of dryness and burning in the oral cavity, the presence of herpetic rashes on the lips and SOPR, cracks, ulcers of the lips and tongue (Figures 1, 2).

Data analysis revealed that the incidence of lesions of the oral mucosa was higher in patients whose disease duration exceeded 30 days and in the early stages of rehabilitation (up to 1 month after COVID-19).

The level of sIgA in the oral fluid during these periods was higher than that of the control group (Table 5).

To determine the relationship of lesions of the oral mucosa with the level of biological markers in the oral fluid (sIgA, elastase and BAPNA-amidase activity), a ROC analysis of the data was performed. It was found that lesions of the oral mucosa were observed at sIgA values > 886.8 mcg/ml (specificity – 94.74%, sensitivity – 47.62%); at the level of BAPNA-amidase activity > 3.1

pcat (specificity – 66.67%, sensitivity – 75%); as well as the level of elastase activity $\leq 90 \times 10^{-5}$ pcat (sensitivity – 90.91% and specificity – 30%).

When determining the diagnostic values of sIgA in patients with COVID-19, based on the ROC analysis, it was found that the sIgA level in the oral fluid above 705.3 micrograms/ml (specificity - 100% and sensitivity – 41.67%), in the presence of concomitant symptoms, it can be assumed that the patient suffered COVID-19 (Figure 3).

5 Conclusion

The presence of confirmed COVID-19 coronavirus infection in patients leads to a significant increase in the level of sIgA and BAPNA-amidase activity in the oral fluid, while at the same time the level of neutrophil elastase activity decreases by about 10 times ($p < 0.001$).

Elimination of the virus and early full-fledged rehabilitation leads to a significant decrease in the level of sIgA in the indicators of the control group. It was revealed that a significant decrease in the level of sIgA occurs during the rehabilitation period from 3 to 6 months ($p = 0.006$), that is, by the 3rd month after the disease, there is a normalization of sIgA indicators in the oral fluid compared with those in the control group ($p > 0.05$).

It was found that lesions of the oral mucosa were more active in the early stages of rehabilitation (up to 1 month after COVID-19), and more than 30 days, while in both cases there was a high level of sIgA in the oral fluid.

The level of sIgA in the oral fluid above 705.3 micrograms/ml in the presence of concomitant symptoms is characteristic of patients who have had COVID-19 infection, thus, the dynamics of the indicators of secretory immunoglobulin sIgA in the oral fluid reveals the possibility of using it as a biological marker for non-invasive rapid diagnosis of coronavirus infection COVID-19.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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