CORRECTION OF DYSBIOSIS AND ELIMINATION OF INFLAMMATION OF THE ORAL CAVITY IN PATIENTS WITH MALIGNANT TUMORS

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Abstract

The article is devoted to the determination of bacterial contamination and antimicrobial protection of the oral cavity in patients with oncology of the oral cavity and also in healthy persons. The study was performed on 38 patients (20 men and 18 women) with stage I-III oral cancer. The average age of patients was 56.5 years (from 33 to 75). To determine the indicators of the norm, a group of norms, including 10 subjects without changes in the oral mucosa, was created. The average age was 29.9 years (26 to 33), both sexes (6 men and 4 women) being represented. Catalase, urease, lysozyme and malonic dialdehyde (MDA) activity was determined. According to the ratio of catalase activity and MDA content, the antioxidant-prooxidant index (API) was calculated and, according to the ratio of relative activities of urease and lysozyme, the degree of dysbiosis was determined by the method of A.P. Levitsky.The study was performed on day 2 of hospitalization and day 14 of treatment. In the experimental group, correction of local immunity was performed.The biochemical studies of oral fluid conducted on patients with tumors of the oral cavity demonstrate the high effectiveness of the proposed local treatment. Thus, under its influence, in the oral cavity of patients, a decrease in the intensity of lipid peroxidation (MDA content), reduction of microbial contamination (urease activity) and of the degree of dysbiosis, with a simultaneous increase in nonspecific antimicrobial protection (lysozyme activity) and antioxidant system, were observed.

Keywords: *dysbiosis, lysozyme, catalase, urease, oral fluid, oncology, oral cavity*.

1. INTRODUCTION

The high frequency of tumors of the oral cavity and oropharynx with involvement in the process of neighboring anatomical structures requires a comprehensive approach for the implementation of extended and combined surgical interventions with replacement of defects with flaps, radiation and chemotherapy. This leads to an increased frequency of local infections, which in turn can lead to failure of postoperative sutures, formation of growths and fistulas [1,2].

In addition, the surgical method of treatment of neoplasms of the mouth and oropharynx remains the main one in the specialized treatment of tumors. The choice of radical surgery is due to the need for rapid and complete removal of the tumor in the oral cavity, and its final histopathological examination. The healing process of postresection wounds is influenced by the state of the microbiota and also by factors of local immunity of the oral cavity.

According to the literature, the frequency of wound infections in the surgical treatment of tumors of the mouth and oropharynx is within significant limits, ranging from 22.7 to 73.0% [3]. The development of infectious complications makes difficult the rehabilitation of patients, leading to deterioration in the quality of life and delaying the start of anticancer therapy.

Lysozyme, as one of the important factors of nonspecific immunity, has found wide application in medicine as a therapeutic and prophylactic agent [4]. Lysozyme-containing drugs and hygiene products for use in dentistry have been developed [5-7].

Therefore, the study of bacterial contamination, lysozyme levels of mixed saliva before and after surgery is relevant for taking preventive measures in the postoperative period.

The aim of the study was to evaluate the antioxidant-prooxidant system of oral fluid, indicators of antimicrobial protection and bacterial contamination of the oral cavity in patients with cancer of the oral cavity and oropharynx, along the stages of surgical treatment

with topical lysozyme applied in the form of a mucosa-adhesive phytogel.

2. MATERIALS AND METHODS

The study was performed on 38 patients (20 men and 18 women) with stage I-III oral cancer, treated at the Podolsk Regional Oncology Center - Department of Head and Neck Tumors. The average age of patients was 56.5 years (from 33 to 75 years). Patients were stage I-III diagnosed with cancer of the tongue (28.9%), cancer of the mucous membrane of the alveolar process of the mandible (28.9%), cancer of the mucous membrane of the bottom of the mouth (11.1%), cancer of the mucous membrane of the lower lip (4.4%), cancer of the cheek mucosa (4.4%), cancer of the mucous membrane of the hard palate (2.2%). Patients with stage IV were not included in the study, they needed only palliative treatment.

25 patients had a primary tumor, 5 patients - recurrence or continuation of tumor growth, which was preceded by chemotherapy (n=2), radiation therapy (n=3), complex treatment (n=3).

Patients did not take antimicrobials 30 days before the study. All patients underwent removal of the tumor with postoperative closure defects by local tissues or regional flaps.

In the postoperative period, patients were divided into two groups of 19 patients each. The experimental group was corrected for local immunity by topical application of mucosa-adhesive phytogel lysozyme. Oral gel applications were done daily for 14 days, twice a day, after meals.

To determine the indicators of the norm, a group of norms including 10 people without changes in the oral mucosa was created. The average age was 29.9 years (26 to 33 years) and was represented by both sexes (6 men and 4 women).

The lysozyme-mucose-adhesive phyto gel contains lysozyme from egg white (5 mg/ml), prebiotic inulin (2%), edible gelatin (1%), mint extract (10%), sodium benzoate (20 mg/ml), sweetener (1 mg/ml), calcium citrate (40 mg/ml), distilled water [9]. Normative and technical documentation (TU, TI) was developed for lysozyme, and permission of the Ministry of Health for its use for prophylactic purposes was obtained [10].

The activity of catalase, urease, lysozyme and malonic dialdehyde (MDA) content was determined [11-14].

According to the ratio of catalase activity and MDA content, the antioxidant-prooxidant index (API) was calculated, and the ratio of relative activities of urease and lysozyme, the degree of dysbiosis was determined according to A.P. Levitsky [15,16].

Saliva collection was performed in the morning, in a centrifuge tube with a funnel, on an empty stomach. For five minutes, the patient spat oral fluid (saliva) into a test tube. It was then centrifuged at 2,500 g for five minutes. Saliva volume was measured, supernatant was collected in clean plastic containers (epindorphs) and frozen until examination. The study was performed on day 2 of hospitalization and day 14 of treatment.

Statistical processing of the obtained data was performed using a mathematical statistical method on a PC using Excel software from Microsoft Office 2003, STATISTICA 5.5 (owned by CNIT VNMU named after M.I. Pirogov, licensed № AXXR910A374605FA) according to Student's criteria. Differences between groups were considered statistically significant at $p<0.05$ [17].

3. RESULTS

Table 1 presents the results of a study on the antioxidant-prooxidant system of oral fluid in patients with tumors of the mouth and oropharynx, before and after treatment. The activity of oral catalase before treatment of both groups of patients (control and experimental) was reduced 2 times, which indicates a low degree of antioxidant protection, as catalase is considered a marker of the state of this system. The analysis showed that topical application of muco-adhesive phytogel lysozyme contributed to a significant increase in catalase activity in the patients from the experimental group by 65.5% ($p_1<0.001$), although its level did not reach normal values. Increased activity of catalase in the oral fluid of patients indicates the pronounced

antioxidant properties of the treatment, in contrast to the control group, where catalase activity almost did not change only after surgical treatment.

Table 1. Indicators of antioxidant-prooxidant system of the oral cavity in patients with oral oncopathology (M ± m), n = 38 before and after treatment (experimental and control group) and without oral pathology (M ± m), n = 10

Groups		Indicators		
		Catalase activity, mcat/l	MDA content, mmol/l	API index
Normal		0.31 ± 0.02	0.12 ± 0.01	25.83 ± 1.12
Experimental	Before treatment	0.15 ± 0.01 p < 0.001	0.32 ± 0.012 p < 0.001	4.68 ± 0.21 p < 0.001
	After treatment	0.24 ± 0.01 p < 0.001 p_1 < 0.001	0.14 ± 0.011 p < 0.001 p_1 > 0.2	17.14 ± 1.09 p < 0.001 p_1 < 0.001
Control	Before treatment	0.12 ± 0.01 p < 0.001	0.38 ± 0.013 p < 0.001	4.78 ± 0.21 p < 0.001
	After treatment	0.16 ± 0.01 p < 0.001 p_1 < 0.001	0.28 ± 0.012 p < 0.001 p_1 < 0.001	10.52 ± 0.89 p < 0.001 p_1 < 0.001

Note: p - indicator of the reliability of the differences from the norm; p_1 - the significance of differences between before and after treatment

The results of determining the content of malonic dialdihydrate (MDA) in the oral fluid of patients presented in Table 1 indicate a significant (2.7 times) increase in this marker in oral oncology (experimental and control groups). The local treatment in the experimental group helped reduce the level of MDA by almost 2.5 times ($p1<0.001$). In the control group, after treatment, the rate exceeded the norm 3 times. MDA is a by-product of lipid peroxidation (LIP). The decrease in its level can be explained by the effective inhibition of the floor, due to the activation of antioxidant protection (from data on catalase activity) after the proposed treatment. The ratio of antioxidant and prooxidant systems, which reflects the antioxidant-prooxidant index (API), was sharply reduced (5.5 times, $p<0.001$) and shifted towards the activation of the prooxidant system in patients with oral tumors, found in the initial study groups. After treatment in the experimental group, API in patients' oral fluid increased 3.7 times, but remained below normal values ($p<0.001$). In the control group, this value also increased, but only 2.2 times.

Table 2 shows the results of determining the indicators of antimicrobial protection and bacterial contamination of the oral cavity in patients with tumors of the oral cavity. The level of activity of lysozyme - the main antimicrobial factor of the oral cavity in the oral fluid of patients with oral cancer at the beginning of the study - was 2.3 times lower than normal ($p<0.001$). After treatment, the activity of lysozyme in the experimental group increased by 65.3% ($p_1<0.001$) compared to its value before treatment. This may indicate stimulation of the production of nonspecific antimicrobial factor of the oral cavity, *i.e.* a well-defined antimicrobial effect of the prescribed local treatment. In the control group, after tumor removal a decrease in lysozyme activity twice ($p_1<0.001$) from baseline and 4 times less than normal ($p<0.001$) was noticed.

The increase in the number of microorganisms in the oral cavity can be judged by the level of

activity of the enzyme urease, which is synthesized by most pathogenic and opportunistic microbiota. According to Table 2, the level of urease activity in the oral fluid of patients before treatment was 3.7 times higher than its normal level ($p<0.001$), which indicates high bacterial contamination of the oral cavity in patients with tumors of the oral cavity. After a course of local treatment, urease activity decreased by 68.1% ($p <0.001$) in the experimental group. This indicates a decrease in bacterial contamination of the oral cavity by increasing the protective properties of the proposed treatment ($p>0.2$). In the control group, urease activity decreased by 44.1% only after surgery.

Table 2. Status of antimicrobial protection and bacterial contamination of the oral cavity in patients with oral oncopathology (M ± m), n=38 before and after treatment (experimental and control group) and without oral pathology (M±m), n=10

Groups		Indicators		
		Lysozyme activity, units/ml	Urease activity, mk-cat/l	Degree of dysbiosis (DD)
Normal		0.112 ± 0.009	0.093 ± 0.008	1.00 ± 0.14
Experimental	Before treatment	0.048 ± 0.004 $p < 0.001$	0.342 ± 0.017 $p < 0.001$	14.71 ± 1.31 $p < 0.001$
	After treatment	0.093 ± 0.008 $p < 0.001$ $p_1 < 0.001$	0.109 ± 0.009 $p > 0.2$ $p_1 < 0.001$	2.55 ± 0.18 $p < 0.001$ $p_1 < 0.001$
Control	Before treatment	0.052 ± 0.004 $p < 0.001$ $p_1 < 0.001$	0.349 ± 0.017 $p < 0.001$	14.65 ± 1.31 $p < 0.001$
	After treatment	0.028 ± 0.002 $p < 0.001$	0.197 ± 0.012 $p < 0.001$ $p_1 < 0.001$	7.04 ± 0.88 $p < 0.001$ p1 < 0.001

Note: p - indicator of the reliability of differences with the norm; p_1 - the significance of differences between before and after treatment

Calculated by the ratio of urease and lysozyme activity, the degree of dysbiosis (DD) of the oral cavity (Table 2) shows that, in individuals with tumors of the oral cavity, this marker was 14.7 times higher than in healthy ones ($p<0.001$). The local treatment contributed to a significant reduction in diabetes in the oral cavity of patients from the study group. However, this value was 2.5 times higher than the normal level ($p<0.001$ and $p_1<0.001$). In the control group, the degree of dysbiosis decreased after surgical treatment, but exceeded the norm by 7 times.

Biochemical studies of the oral fluid of patients with tumors of the oral cavity demonstrate the high effectiveness of the proposed local treatment. Under its influence in the oral cavity of patients with oncopathology, decrease in the intensity of lipid peroxidation (MDA content), decrease in microbial contamination (urease activity) and the degree of dysbiosis with simultaneous increase in nonspecific antimicrobial protection (catalase and API activity) were found.

4. DISCUSSION

The oral cavity provides favorable conditions for the development of beneficial, pathogenic and opportunistic microorganisms. Microorganisms that grow in the oral cavity depend on many factors of the macroorganism, environmental impact, social and behavioral habits. Intensive accumulation and dissemination of pathogenic microorganisms creates the

preconditions for the development of inflammatory processes, formation of autoimmune processes, chronic diseases of various organs and systems [18].

Sufficient significance of dysbiosis in the course of diseases of the gastrointestinal tract has been described [19], as well as the microbiological picture of the oral cavity and its correction in *diabetes mellitus* [20]. There is also data on the composition of the microbiota about tumors of the oral cavity [21]. However, no data is available on how this affects healing.

A feature of our study was the study of some indicators of nonspecific immunity and its correction in the postoperative period. After tumor removal, the level of lysozyme decreases, which adversely affects wound healing, while the proposed local treatment significantly increases the level of lysozyme.

5. CONCLUSIONS

1. In patients with cancer of the mouth and oropharynx reduced catalase and antioxidant-prooxidant index ($p<0.001$) were recorded.

2. This indicates a violation of nonspecific resistance. High levels of urease and the degree of dysbiosis ($p<0.001$) indicate bacterial contamination of the oral cavity in patients with oncopathology. Decreased level of oral lysozyme ($p<0.001$) indicates a violation of local immunity.

3. Topical application of muco-adhesive phytogel lysozyme in patients with tumors of the oral cavity in the postoperative period indicates its antimicrobial and antioxidant efficacy, increasing local immunity.

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