BACTERIAL INSEMINATION AND ANTIMICROBIAL PROTECTION OF THE ORAL CAVITY AND ITS IMPORTANCE IN THE GENESIS OF CANCER IN THIS AREA

Anna KUSHTA[1]

[1]PhD, MD, Associate Professor, National Pirogov Memorial Medical University, Vinnytsya, Ukraine
Corresponding author: Anna Kushta; e-mail: dr_anna9@ukr.net

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 people, as a review of the literature on this issue. The aim of our 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, comparatively with healthy individuals. The study was conducted on 30 subjects, including 20 patients with stage I-III oral cancer and 10 without cancer. The former were patients with cancer of the tongue (28.9%), of the mucous membrane of the alveolar process of the mandible (28.9%), of the mucous membrane of the bottom of the mouth (11.1%), of the mucous membrane of the lower lip (4.4 %), of the cheek mucosa (4.4%), of the mucous membrane of the hard palate (2.2%).The biochemical studies of oral fluid conducted in patients with oral oncopathology showed an increase in the intensity of lipid peroxidation (MDA content), high levels of microbial contamination (urease activity) and some degree of dysbiosis, with a simultaneous decrease in nonspecific antimicrobial protection (lysozyme activity) and antioxidant system (catalase and API activity). The results indicate a decrease in the local immune response. Analyzing the obtained results in the postoperative period in patients with oncopathology of the oral cavity, it is necessary to correct the local immune response, thus increasing non-specific antimicrobial protection for better wound healing and for avoiding postoperative complications.

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

1. INTRODUCTION

Smoking and alcohol use are common risk factors for oral and pharyngeal cancer. However, in recent years, there is evidence that the microbiota of the oral cavity and its changes may play a direct role in the development of cancer in this location [1-4]. It is believed that the microbiota has several effects that promote cancer, namely: 1) stimulation of chronic inflammation by bacteria (inflammatory mediators produced in this process cause or facilitate cell proliferation, mutagenesis, oncogene activation and angiogenesis); 2) influence of bacteria on the pathogenesis of cancer through the influence on cell proliferation (through the activation of NF-Kb and inhibition of cellular apoptosis); 3) production by bacteria of substances that act as carcinogens [5]. The high frequency of tumors of the oral cavity and oropharynx involving neighboring anatomical structures requires a comprehensive approach to advanced and combined surgery, with replacement of the defects with flaps, radiation and chemotherapy, which leads to increased incidence of local infections, which in turn can lead to sutures, overgrowth and fistula formation, and phlegmon development [6,7]. The occurrence of infectious complications disrupts the rehabilitation of patients, leads to a deterioration in quality of life and delays the start of anticancer therapy. According to literature, the frequency of wound infections in the surgical treatment of tumors of the mouth and oropharynx lies within significant limits, ranging from 22.7 to 73.0% [8]. The development of acute and chronic inflammatory processes (caries, gingivitis, periodontitis, etc.) causes changes in the microbial landscape, dysbiosis, thus weakening local immunity in this area. To date, there are a number of works in which the evidence base confirms that certain microorganisms may possibly promote the development of tumor processes, in particular, squamous cell carcinoma of the mucous membranes of the mouth and pharynx [9-12]. However, among the large number of bacteria, their end products are important.The aim of our

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, comparatively with healthy individuals.

2. MATERIALS AND METHODS

The study was conducted on 30 subjects, including 20 patients with stage I-III oral cancer and 10 people without cancer. The average age of patients treated at the Podolsk Regional Oncology Center in the Department of Head and Neck Tumors was 56.5 years (from 33 to 75 years). The men-to-women ratio was 12: 8. The patients were diagnosed with: cancer of the tongue (28.9%), of the mucous membrane of the alveolar process of the mandible (28.9%), of the mucous membrane of the bottom of the mouth (11.1%), of the mucous membrane of the lower lip (4.4 %), of the cheek mucosa (4.4%), of the mucous membrane of the hard palate (2.2%). Distribution of patients by stages is presented in Table 1.

Table 1. Distribution of patients by stages and TNM system included in the study, n=20

Stage of disease	Cancer of the tongue, *n*	Cancer of the mucous membrane of the alveolar process of the mandible, *n*	Cancer of the mucous membrane of the bottom of the mouth, *n*	Cancer of the mucous membrane of the lower lip, *n*	Cancer of the cheek mucosa, *n*	Cancer of the mucous membrane of the hard palate, *n*
I stage	1	2	-	-	1	-
II stage	3	2	2	2	2	1
III stage	2	2	-	-	-	-

In 13 patients there was a primary tumor, in 7 patients - recurrence or continuation of tumor growth, preceded by chemotherapy (n=2), radiation therapy (n=2), complex treatment (n=3).

The control group included 10 persons, mean age - 29.9 years (26 to 33 years) and was represented by both sexes (6 men and 4 women).

Catalase activity, malonic dialdehyde content, antioxidant-prooxidant index, which are indicators of the antioxidant-prooxidant system of oral fluid, were determined in all patients. Indicators of antimicrobial protection and bacterial contamination of the oral cavity in patients were also determined, namely lysozyme activity, urease and degree of dysbiosis. The degree of dysbiosis of the oral cavity was determined using the enzymatic method of examination of the oral fluid. Lysozyme activity in saliva correlates with the level of antimicrobial factors of the macroorganism, while urease is produced by most bacteria, but not by somatic cells. Assessment of its activity can be done to determine the degree of contamination of the oral cavity with microorganisms. Saliva collection was performed in the morning, in a centrifuge tube with a funnel, on an empty stomach. For five minutes, the patient spat the oral fluid (saliva) into a test tube, which was then centrifuged at 2,500 g for five minutes. Saliva volume was measured, the supernatant being collected in clean plastic containers (epindorphs), then frozen for examination.

In patients with onopathology of the oral cavity, saliva collection was performed on the 2nd day of hospitalization before operation. Surgical treatment included removal of the tumor with closure of the postoperative defect by local tissues or regional flaps.

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 MI Pirogov, licensed № AXXR910A374605FA), according to Student's criteria. Differences between groups were considered statistically significant at $p < 0.05$ [13].

3. RESULTS

Table 2 presents the results of a study on some indicators of the antioxidant-prooxidant system of oral fluid in patients with oncopathology of the oral cavity.

The activity of catalase in the oral fluid of patients before treatment was reduced 2 times, which indicates a low degree of antioxidant protection, as catalase is considered a marker of the state of this system.

Table 2. Indicators of antioxidant-prooxidant system of the oral cavity in patients with oncopathology of the oral cavity (M±m), n=20

Indexes	Catalase activity, mcat/l	MDA content, mmol/l	API index
Norm (healthy people)	0.31 ± 0,02	0.12 ± 0.01	25.83 ± 1.12
Patients with oncopathology	0.15 ± 0,01 p < 0,05	0.32 ± 0.012 p < 0,05	4.68 ± 0.21 p < 0,05

Note: p - indicator of the reliability of differences with the norm

The results of determination of the content of malonic dialdihydrate (MDA) in the oral fluid of patients presented in Table 2 indicate a significant (2.7 times) increase in this marker, in the oncopathology of the oral cavity. MDA is a secondary product of lipid peroxidation (LPO), so that its increasd level can be explained by a decrease in antioxidant protection (from data on catalase activity).

The ratio of antioxidant and prooxidant systems, which reflects the antioxidant-prooxidant index (API), was sharply reduced (5.5 times, p <0.05) and shifted towards the activation of the prooxidant system in patients with oral cancer.

Table 3 shows the results obtained when determining the indicators of antimicrobial protection and bacterial contamination of the oral cavity in patients. The level of activity of lysozyme - the main antimicrobial factor of the oral cavity in the oral fluid of patients with oral cancer - was 4 times lower than the normal level (p <0.05). This may indicate a decrease in nonspecific antimicrobial factor of the oral cavity, *i.e.* violation of local immunity of the oral cavity.

Table 3. Antimicrobial protection and bacterial contamination of the oral cavity in patients with oncopathology of the oral cavity (M±m), n=20

Indexes	Lysozyme activity, units / ml	Urease activity, mk-cat / l	Degree of dysbiosis (DD)
Norm (healthy people)	0,112 ± 0,009	0,093 ± 0,008	1,00 ± 0,14
Patients with oncopathology	0,028 ± 0,002 p < 0,05	0,342 ± 0,017 p < 0,05	14,71 ± 1,31 P < 0,05

Note: p - indicator of the reliability of differences with the norm

The increased number of microorganisms in the oral cavity can be judged by the level of activity of enzyme urease, which is synthesized by most pathogenic and opportunistic microbiota. As shown in Table 3, the level of urease activity in the oral fluid of patients with oral oncology was 3.7 times higher than the normal level of this indicator (p<0.05), which evidences a high bacterial contamination of the oral cavity in patients and also a decrease in protective properties.

Calculated by the ratio of the relative activity of urease and lysozyme, the degree of dysbiosis (DD) of the oral cavity (Table 3) shows that, in persons with oral oncopathology, this marker was 14.7 times higher than in healthy individuals (p<0.05).

Consequently, biochemical studies on oral fluid in patients with oral oncopathology showed high bacterial contamination, increased microbial contamination, a high degree of dysbiosis and reduced nonspecific antimicrobial protection (lysozyme activity).

4. DISCUSSION

Nowadays, it is believed that bacterial contamination of the oral cavity is compositionally and functionally associated with the mutational changes produced by oral cancer [9]. This is due to damage to the mucosa and hyperproliferation of epithelial cells and inflammation [14,15]. According to the literature of the field, the microbiota of the oral cavity includes representatives of more than 700 bacterial species [16]. Changes in their ratio lead to dysbiosis. There is a direct relationship between the microbial landscape of the oral cavity and the metabolic processes developed in the human body and local immunity.

The biochemical studies of oral fluid conducted on patients with oral oncopathology showed an increase in the intensity of lipid peroxidation (MDA content), high levels of microbial contamination (urease activity) and degree of dysbiosis, with a simultaneous decrease in nonspecific antimicrobial protection (lysozyme activity) and antioxidant system (catalase and API activity). The results indicate a decrease in the local immune response.

Analysis of the obtained results in the postoperative period in patients with oncopathology of the oral cavity evidenced the necessity to correct the local immune response, to increase non-specific antimicrobial protection for better wound healing and avoid postoperative complications.

5. CONCLUSIONS

1. The enzymatic method of oral fluid examination in patients with oncopathology of the oral cavity is informative and easy to pick up material for research.
2. Patients with oral and oropharyngeal cancer have reduced catalase levels and a low antioxidant-prooxidant index ($p < 0.05$), which indicates violation of nonspecific resistance, in particular, the presence of cellular mechanisms of damage.
3. High levels of urease and of dysbiosis ($p<0.05$) indicate bacterial contamination of the oral cavity in patients with oncopathology.
4. Decreased level of lysozyme in oral fluid ($p<0.05$) indicates a violation local immunity.
5. The obtained results of biochemical parameters of oral fluid should necessarily take into account correction of local immunity and reducing bacterial contamination to improve the regenerative properties of tissues.

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