

# EVALUATION OF TOTAL OXIDANT STRESS AND TOTAL ANTIOXIDANT CAPACITY IN SMOKING AND NON-SMOKING PATIENTS UNDERGOING SURGICAL EXTRACTION OF THIRD MOLARS

Maciej Wrzoł<sup>1</sup>, Tadeusz Morawiec<sup>1</sup>, Jolanta Zalejska-Fiolka<sup>2</sup>, Patryk Kownacki<sup>1</sup>, Ewa Chwalińska<sup>2</sup>, Alicja Hampel<sup>2</sup>, Rafał Rój<sup>3</sup>, Jiří Stránský<sup>4</sup>, Adam Pasz<sup>4</sup>, Iwona Niedzielska<sup>5</sup>

<sup>1</sup>Department of Oral Surgery, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Bytom, Poland

<sup>2</sup>Department of Biochemistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Zabrze, Poland

<sup>3</sup>Department of Prosthetic Dentistry, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Bytom, Poland

<sup>4</sup>Department of Oral and Maxillofacial Surgery, University Hospital Ostrava, Ostrava, Czech Republic

<sup>5</sup>Department and Hospital of Craniomaxillofacial Surgery and Oral Surgery, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Poland

## ABSTRACT

**INTRODUCTION:** The increased rate of reactive oxygen species (ROS) or failure of antioxidant mechanisms leads to the oxidative stress, which is a etiopathological factor of many systemic diseases and may have an adverse effect on the healing process. Saliva, equipped with antioxidative mechanisms is an important defense against ROS.

**OBJECTIVES:** The aim of the study was to evaluate the antioxidant profile parameters of saliva (total oxidant stress and total antioxidant capacity) in smoking and non-smoking patients undergoing surgical extraction of third molars.

**MATERIAL AND METHODS:** The study was performed in a group of 30 patients who underwent a surgical extraction of partly impacted third molars. A sample material for testing in the form of saliva was collected from the patients before the surgery and 7 to 10 days after surgical treatment. The acquired material was assessed taking into account its antioxidant profile: TOS (total oxidant stress), and TAC (total antioxidative capacity).

**RESULTS:** Statistically after the surgical treatment in the group of non-smokers, the TAC level was higher ( $p = 0.042$ ), the TOS level in the group of male smokers was statistically higher ( $p < 0.05$ ). In non-smoking male group the TAC level was statistically higher ( $p = 0.046$ ).

**CONCLUSIONS:** It was observed an impact of surgical treatment in the oral cavity on the antioxidative – oxidative status of saliva. It was found beneficial in the group of non-smokers and smoke free men (TAC increased) and unfavorable in the group of male smokers (TOS increased).

**KEY WORDS:** oral surgery, reactive oxygen species, total antioxidant capacity, total oxidant stress, wisdom teeth surgery.

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**ADDRESS FOR CORRESPONDENCE:** Maciej Wrzoł, Department of Oral Surgery School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, 17 Plac Akademicki Str., 41-902 Bytom, Poland, e-mail: [mwrzol@sum.edu.pl](mailto:mwrzol@sum.edu.pl)

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## INTRODUCTION

The oral cavity, due to repeated contact with potentially dangerous exogenous factors, is the place of formation of reactive oxygen species (ROS) or free radicals (FR) playing an important role in both physiological and pathological processes. Factors inducing the synthesis of free radicals include exogenous factors (such as ionizing radiation, UV, water and air pollution, cigarette smoke, certain drugs) and endogenous factors, i.e. physiological processes with one-electron transfer (respiratory chain) or inflammatory processes. Reactive oxygen species include the superoxide anion radical ( $O_2\cdot$ ), hydroperoxyl radical ( $HO_2\cdot$ ), hydroxyl radical ( $HO\cdot$ ), and alkoxy radical ( $RO\cdot$ ).

Proper cellular processes mostly depend on the oxidative–antioxidative balance. One of the conditions of homeostasis is the balance between the rate of production of ROS and the activity of antioxidant systems. The increased rate of production of ROS or failure of antioxidant mechanisms leads to oxidative stress, which in turn is an etiopathological factor of many systemic diseases and may have an adverse effect on the healing process.

Saliva, equipped with antioxidative mechanisms, is an important defense against ROS. The main components of saliva involved in the defense against free radicals are low molecular weight antioxidants, i.e. glutathione, ascorbate, uric acid, creatinine, bilirubin, tocopherols, carotenoids, coenzyme Q, and antioxidant enzymes, i.e. superoxide dismutase, catalase, glutathione peroxidase and so called prevention antioxidants, i.e. albumin, transferrin, lactoferrin, ceruloplasmin, haptoglobin and hemopexin [1, 2]. The sources of non-enzymatic antioxidants are vitamins and minerals, especially vitamins A, C and E, carotenoids and phenolic compounds. The richest sources of these substances are fruits and vegetables and their products. Berries are rich in anthocyanins and citrus fruits are the source of flavanones. Significant quantities of antioxidant compounds are provided by infusions such as coffee, tea, red wine, beer and herbs and spices [3]. They are also present in bee glue (propolis) and oil from the tea tree [4].

Current clinical trials are mainly focused on evaluation of the antioxidant profile of patients during various systemic disorders, periodontopathy or active caries. One can find in the literature some studies proving that the presence of periodontopathogens in the oral cavity increases the number of ROS. This oxidative profile causes damage of collagen and periodontal tissues. Other studies suggest that the consequences of local oxidative stress may be increased impact of carcinogenic factors in the oral cavity and systemic increase of atherogenesis. Reviewing the available literature one can find some reports about preventive measures. Selected formulations applied locally can have an impact on the reduction of oxidative stress as it was proved in the example of vitamin E, which being a precursor of antioxidants

protects against ROS and particularly  $H_2O_2$  [5-8]. Based on this knowledge the manufacturers add to the preparations such as toothpastes and mouthwashes substances having antioxidants properties. In addition, they are more and more applied as dietary supplements because the preliminary results of some studies suggest that they may have a beneficial effect on the prevention of cancer and ischemic heart disease (IHD) [9, 10].

The third molars, commonly called the eighths or wisdom teeth, are the teeth which are most often impacted or are not erupted during the physiological period of time. Changing the diet from hard to more soft had an impact on the gradual reduction of the size of the maxilla and the mandible, which causes that the third molars are impacted [11].

Extraction of impacted and partly impacted third molars is often performed because of inflammation associated with difficult eruption or is connected with orthodontic reasons. One can divide the healing of bone defects after tooth extraction into 4 phases: inflammation, soft callus formation, hard callus formation, and remodeling. The first phase or inflammatory lasts about 7 days. At this time the body's response is a reaction in which the concentration of acute phase proteins induced by TNF and interleukins 1 and 6 (IL-1, IL-6) in blood increases [12].

## OBJECTIVES

The aim of the study was to evaluate the antioxidant profile parameters of saliva (total oxidant stress and total antioxidant capacity) in smoking and non-smoking patients undergoing surgical extraction of third molars.

## MATERIAL AND METHODS

The study was performed in 30 patients who underwent surgical extraction of partly impacted third molars. They were divided into 6 groups according to their smoking habits and sex as below:

1. The group of non-smoking men and women: "N-S" – 20 patients\*;
2. The group of non-smoking women: "N-S (W)" – 11 patients;
3. The group of non-smoking men: "N-S (M)" – 9 patients;
4. The group of smoking women and men: "S" – 10 patients\*;
5. The group of female smokers: "S (W)" – 5 patients;
6. The group of male smokers: "S (M)" – 5 patients.

\*Group 1 – "N-S" – contains all non-smoking patients (patients from groups 2 and 3).

\*Group 4 – "S" – contains all smoking patients (patients from groups 5 and 6).

During qualification for surgical treatment thorough clinical and radiological examination was carried

out, including a detailed interview about the clinical burden of the patient and applied medication, age and sex and used substances, especially smoking cigarettes, and the following inclusion/exclusion criteria were applied.

**Inclusion criteria:** healthy patients qualified for treatment in dental surgery, patients burdened with systemic diseases at the level which allows surgery in the field of dental surgery to be performed, smoking patients, non-smoking patients.

**Exclusion criteria:** patients during diagnostics and treatment of tumors of the head and neck, pregnant women, patients burdened with systemic diseases which make it impossible to perform the treatment in an out-patient clinic.

All patients underwent the surgical treatment using the same surgical technique. After local anesthesia with 2% lidocaine with noradrenaline and after angle cutting a mucoperiosteal flap was separated. Then, after removing a small amount of the outer lamina dura of the bone they underwent extraction of the whole tooth or tooth after crown and root separation. Then the wound was managed with nylon surgical suture 3.0.

The sample material for testing in the form of saliva was collected from the patients. Saliva was collected using Salivettes (Sarstedt) before surgical treatment and 7 to 10 days after surgical treatment. The volume of the collected saliva was between 1 and 3 ml. The patient, on the day of surgical treatment and follow-up visits, was at least 30 minutes after a meal and after cleansing of the oral cavity with a toothbrush and toothpaste. The collection of saliva was performed by chewing of the cotton cartridge of the Salivette for 2 minutes and then placing it in a Salivette container. The Salivette was then transported to the laboratory of the Department of Biochemistry of the Medical University of Silesia where the material was centrifuged in an automatic test tube centrifuge (2200 rpm for 10 minutes). The acquired material was assessed taking into account its antioxidant profile: TOS (total oxidant stress), and TAC (total antioxidative capacity) using the PerkinElmer VICTOR-X3 reader.

## BIOCHEMICAL EXAMINATION

### TOTAL OXIDANT STRESS MARKING

The method is based on the oxidation of iron ions (II) to iron ions (III) in acidic media. Then the iron ions (III) form a colored complex with xylenol orange up to blue-purple color.

Absorbance reading was done with a 560 nm filter on the PerkinElmer VICTOR-X3 reader.

The concentration was calculated on the basis of the standard curve using  $H_2O_2$  as a standard.

The values are presented in  $\mu\text{mol/g}$  of protein.

### TOTAL ANTIOXIDATIVE CAPACITY MARKING

The method is based on the discoloration of oxidized ABTS (green) under the influence of antioxidants contained in the tested material.

Absorbance reading was done with a 650 nm filter on the PerkinElmer VICTOR-X3 reader.

The concentration was calculated from the standard curve using Trolox as a standard.

The values are presented in  $\text{mmol/g}$  of protein [13-16].

## STATISTICAL ANALYSIS

The results of the study were analyzed in relation to the sex of patients and the use of nicotine. All statistical analyses were performed using the STATISTICA 7.0 program (StatSoft, Inc., Tulsa, OK., USA). To determine an adequate statistical test for the trials, the Kolmogorov-Smirnov test was used. In cases where the test results indicated that the test sample fulfilled the conditions for normal distribution and homogeneity of variance (Levene test), the ANOVA test (comparisons for three groups) was used for internal and external group comparisons. Then a post hoc test was applied. If the Levene test result showed equality of variance, the Tukey test was used; otherwise the Games-Howell test was used. In the case of a different distribution than normal, a nonparametric alternative to the analysis of variance in the form of the Kruskal-Wallis test was used. This test does not require assumptions about normal distribution and homogeneity of variance. To compare two independent groups, the Mann-Whitney  $U$  test was used in the absence of a normal distribution and Student's  $t$  test for normal distribution. To compare the dependent variables, the Wilcoxon test was used. Statistical significance was determined for  $p$  values  $< 0.05$ .

## RESULTS

The TAC level difference before and after surgery was statistically significant in the group of non-smokers and non-smoking men ( $p < 0.05$ ). The TOS level difference before and after surgery was statistically significant in the group of male smokers ( $p < 0.05$ ). In the other groups it did not show any statistical significance (Table 1).

In the group of non-smokers the TOS level difference before and after surgery was not statistically significant but the TAC level was significantly higher ( $p = 0.042$ ) after the surgical treatment (Figure 1).

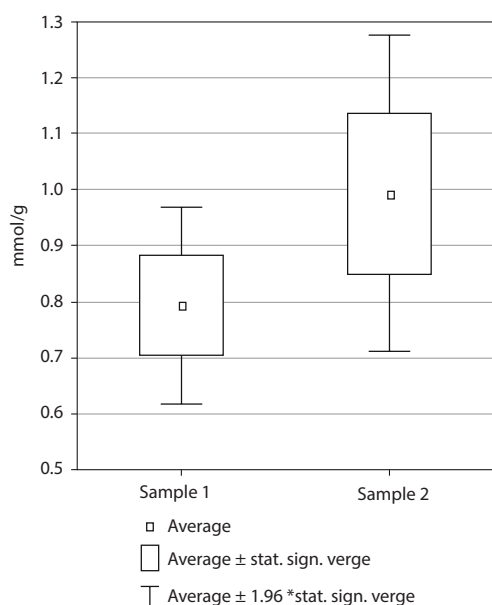
Compared to the group of smokers the TOS and TAC level differences before and after surgery were not statistically significant.

The TOS level in the group of male smokers was significantly higher ( $p < 0.05$ ) after the surgical treatment (Figure 2).

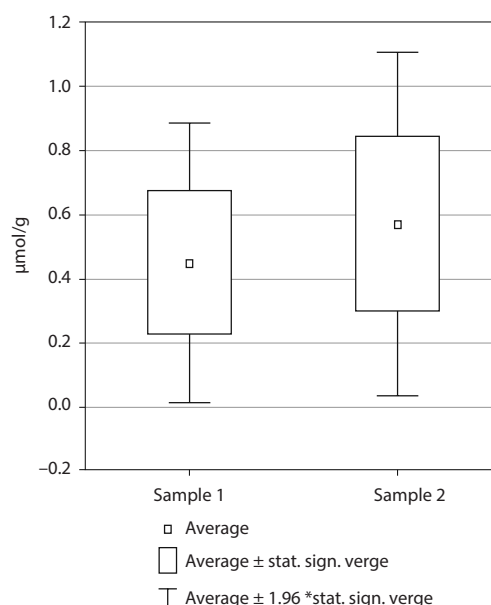
**TABLE 1.** Mean, standard deviation (SD) and statistical significance ( $p$ ) for TAC and TOS before (1) and after (2) surgery for each group

Marker Group	TAC 1 (mmol/g)		TAC 2 (mmol/g)		$p$ < 0.05	TOS 1 ( $\mu\text{mol/g}$ )		TOS 2 ( $\mu\text{mol/g}$ )		$p$ < 0.05
	Average	SD	Average	SD		Average	SD	Average	SD	
N-S	0.79349	0.39983	0.99125	0.64342	0.042	0.91467	1.28200	0.93392	1.64713	0.65
N-S (W)	0.72927	0.34888	0.90641	0.65933	0.375	1.02066	1.53098	1.23404	2.20004	0.59
N-S (M)	0.87198	0.46361	1.09494	0.64640	0.046	0.78512	0.96912	0.56710	0.34351	0.95
S	0.81728	0.48257	0.75746	0.44314	0.57	1.58299	1.72699	1.36110	1.49234	0.87
S (W)	0.69516	0.28276	0.56436	0.25770	0.13	2.71686	1.80232	2.15030	1.75361	0.68
S (M)	0.93940	0.63776	0.95057	0.53124	0.89	0.44913	0.49816	0.57191	0.61536	0.049

N-S – non-smokers, S – smokers, M – men, W – women



**FIGURE 1.** The level of TAC before (sample 1) and after (sample 2) surgical treatment in the group of non-smokers



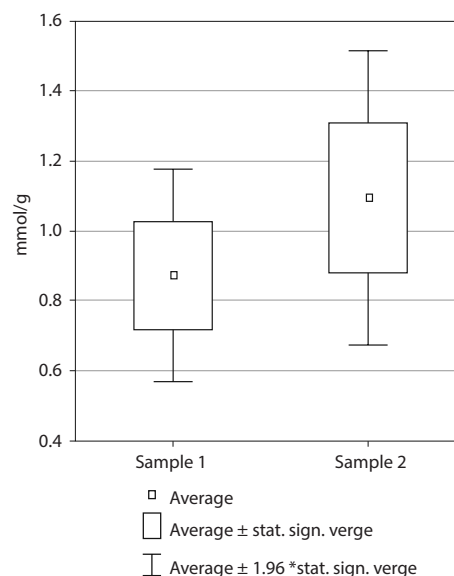
**FIGURE 2.** The level of TOS before (sample 1) and after (sample 2) surgical treatment in the group of male smokers

In male non-smokers group the TAC level was significantly higher ( $p = 0.046$ ) after the surgical treatment (Figure 3).

## DISCUSSION

Due to the fact that an excessive level of oxidative stress can have a negative impact on the healing processes and a normal course of the cellular processes, to a significant extent, depends on maintaining an oxidative–antioxidative balance, it seems to be expedient to evaluate the parameters of oxidative stress after the performed operative procedure within the area of the oral cavity [1, 2].

Ullmann *et al.* [17] evaluated certain saliva parameters, inter alia activity of SOD and the level of TAC, in patients before and after a major surgical procedure. They observed variability of the oxidative–antioxidative status of the saliva by the increased values of TAC and increased activity of SOD in patients before and after



**FIGURE 3.** The level of TAC before (sample 1) and after (sample 2) surgical treatment in the group of non-smoking men

the operational procedure in comparison with a control group. The authors place hope in the evaluation of certain saliva parameters as a tool for monitoring the preoperative and postoperative periods. So, the major surgical procedure is not without any impact on the saliva oxidative–antioxidative status. With regard to the above, it has been of importance for us to determine the nature of the operative procedure of tooth extraction of an impacted third lower molar.

The operative procedure of extracting the impacted and partly impacted third lower molars can be included in so-called minor surgical procedures [18]. Nevertheless, it is the invasive procedure, which consists in an intervention not only in the areas of the soft tissues, but also in the hard tissues – maxillary alveolar bone or the alveolar part of the mandibula. The period which this study focuses on is the so called first healing phase: an inflammatory phase, which lasts about 7 days. Within this period the permeability of the vascular endothelium increases, which results in frequent swelling of the operated area. It can also be accompanied by painful conditions. In some cases, there can occur trismus and a periodical increase of the body temperature. In the normally healed wounds some free radicals, such as hydrogen peroxide and superoxide radical anion, can act as indicators of key processes related to healing that include normal cell mobility, cytokinin activity or angiogenesis. In the inflammatory phase, in the blood there are increases in concentrations of the acute-phase proteins induced by TNF- $\alpha$  and interleukins 1 and 6 (IL-1, IL-6). Both oxygen deficiency and hyperoxia of the wound area can result in increased production of WR, which disturbs the oxidative–antioxidative balance. The excessive production of WR exceeds the beneficial activity and causes additional damage of the tissue [12, 18].

The increased level of free radicals is ascribed to more frequent occurrence of periodontopathies, hindered tissue healing, and even an increased risk of oral squamous cell carcinoma. The studies that evaluate an oxidative–antioxidative potential of the non-stimulated saliva have shown that in patients who suffer from chronic periodontitis there can occur a decrease of the local efficiency of the antioxidative mechanisms while at the same time the activity of RTF is increased, which results from stimulation of neutrophils and macrophages in the periods of exacerbation of the disease. The antioxidative potential of the saliva, blood serum, and gingival crevicular fluid shows heterogeneous values. The local antioxidative capability seems to last as long as the periodontopathy lasts. It can predispose to the further, more dynamic course of the periodontal disease. The effects of oxidative stress can also include widening of the impact of carcinogenic factors in the oral cavity and a systemic intensification of the atherogenesis process [7, 19].

In a study conducted in patients who suffer from oral lichen planus there has been evidenced a higher level

of thiobarbiturates (TBARS) as an indicator of increased oxidative stress and a decrease in the value of TAC compared to the control group – patients who do not suffer from this disease [20]. The results of the study conducted by Babaee *et al.* demonstrated statistically significantly higher values of MDA and lower values of TAC in patients who suffer from recurrent aphthous stomatitis compared to the control group, which indicates the increased oxidative stress and decreased antioxidative capacity of saliva in these patients [21].

In the literature, there also appear reports on prophylactic effects. Topical use of vitamin preparations can contribute to a reduction of oxidative stress, which has been evidenced in the example of vitamin E, which as the anti-oxidant precursor protects against RTF, in particular H<sub>2</sub>O<sub>2</sub> [4, 7, 8]. Błochowiak *et al.* [22] studied concentrations of the products of lipid peroxidation in patients with mandibular fractures. They proved that the antioxidants used at that time accelerate the union of fractured bones and eliminate the disadvantageous effects of RTF. In turn, Turk *et al.* [23] evidenced an advantageous impact of vitamin E for a condition of the bone tissue, administered both in the early and late periods of fracture treatment. The changes of the selected indices of oxidative stress obtained from the saliva and blood during the described therapy are of a temporary nature – the values return to the initial values after completing the treatment.

Shirzaiy *et al.* [24] studied the antioxidative capacity of saliva in healthy, non-smoking patients who suffer from chronic periodontitis prior to and after the periodontological treatment. The antioxidative capacity of the saliva was statistically significantly higher in patients who had completed the periodontological treatment. Similar results were obtained by Yang *et al.* [25], who studied the influence of removing tartar while using ultrasonic scaling. The activity of SOD increased more than two-fold in patients who irregularly visited a dentist (less than once per year). In patients who visit a dentist on a regular basis the value of TAC after scaling was statistically significantly higher than in patients who visit a dentist erratically.

Our research showed that the levels of TAC and TOS significantly changed after surgical procedures.

The TAC values in the non-smoking group and non-smoking men group increased. In the case of TOS, its level increased in the group of smoking men. A conclusion can be presented that after surgical intervention the antioxidative potential increased in the non-smoking and non-smoking men groups. In the case of the smoking men group the level of oxidative stress increased in the post-surgery period.

Saliva is a biological fluid, so it is important to assess whether the concentration of MDA, which is the main product of lipid peroxidation, can be an indicator of oxidative processes in the oral cavity. In a study performed in 2015 with a group of 10 patients aged be-



tween 10 to 64, there concentration of malondialdehyde in the saliva was observed. The material was sampled before the surgical procedure of impacted third lower molar extraction and 7-10 days after the intervention. The studied group consisted of 5 non-smoking patients and 5 smokers. The level of MDA decreased after the surgical procedures in 8 of 10 studied patients. In the non-smokers, the elimination of MDA after the surgical intervention was more significant than in the declared smokers group [26].

In the study of Kurku *et al.* [27], during which the levels of MDA, GPx and NO in the saliva prior and after smoking a cigarette were measured, it was found that the level of MDA in smokers was higher prior to and after smoking a cigarette ( $p < 0.05$ ) than in the control group (non-smokers), and the level of glutathione peroxidase (GPx) was lower than in the control group. The level of NO in the saliva was higher after having a cigarette than before smoking and it was higher in comparison with the control group ( $p < 0.05$ ).

Mojtaba *et al.* [28] observed a lower concentration of TAC in smokers in comparison with the non-smokers and Hamid-Raza *et al.* [29] also evidenced a lower activity of superoxide dismutase and GPx in smokers in comparison with non-smokers. In their study Giuca *et al.* [30] found a significant decrease of the activity of GPx in the smokers group ( $p < 0.05$ ), but the impact of the activity of SOD was not determined. Depending on sex, a significant drop of GPx activity was noted in men from the smokers' group ( $p < 0.05$ ), while in the women sample no significant differences in the enzymatic activity were determined. Furthermore, among the ex-smokers there were no significant differences in the values of GPx between those who had smoked less than ten years and those had not smoked for more than ten years. So, the cigarette smoke may weaken detoxification of hydrogen peroxide by lowering the activity of GPx, so increasing the level of oxidative stress in the group of smoking men.

Research presented above indicates an impact of smoking cigarettes on the oxidative-antioxidative status of the saliva as disadvantageous. In turn Giuca *et al.* [30] proved that the changes in the activity of GPx depend not only on smoking cigarettes, but also on the patient's sex. In our study, after the conducted surgical procedure in the group of smoking men we observed an increase of the TOS level.

An advantageous impact on the oxidative-antioxidative balance is observed during the course of the treatment of the pathological conditions of the oral cavity, for instance after tartar removal in persons with periodontitis. In our study, after the executed operative procedure in non-smokers and the group of non-smoking men, we observed an increase of total antioxidative capacity of the saliva. It demonstrates an improvement of the oxidative-antioxidative status in these groups after the surgery.

## CONCLUSIONS

We observed an impact of the surgical treatment in the oral cavity on the antioxidative-oxidative status of saliva. In the group of non-smokers and non-smoking men we found a beneficial impact due to the increase of the level of TAC, but in the group of male smokers this impact turned out to be unfavorable due to increase of the level of TOS. The results of our study are similar to the research results of other authors evaluating the impact of smoking on the oxidative-peroxidative status of saliva and identified it as a negative one.

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