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Oxidative Stress in the Oral Cavity before and After Prosthetic Treatment

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Abstract

BACKGROUND: Metal ions emitted from dental alloys may induce oxidative stress leading to numerous pathological changes. Lipid peroxidation may cause disturbance of structure and function of cell membranes, apoptosis, autophagy, and formation of potentially mutagenic compounds. Products of interaction between reactive oxygen species and biomolecules may be used for evaluation of oxidative stress level.

AIM: The aim of this study was to evaluate the influence of the prosthetic dental treatment with metal ceramic restorations on the level of oxidative stress in the oral cavity.

MATERIALS AND METHODS: Metal ceramic crowns with copings fabricated by direct metal laser sintering were produced for 35 patients. CoCr dental alloy EOS CobaltChrome SP2 (EOS) was used. Non-stimulated and stimulated saliva samples were collected from the patients before and after the prosthetic treatment. For evaluation of oxidative stress concentration of 8-isoPGF2-alpha was measured by liquid chromatography tandem mass spectrometry. For statistical processing, non-parametric Wilcoxon signed-rank test and Mann–Whitney test were applied.

RESULTS: The concentration of isoprostane 8-isoPGF2-alpha in non-stimulated saliva was lower 2 h after fixing the crowns compared to the initial level and statistically significant difference was observed. On the 7th day the concentration of isoprostanes remained significantly lower than the initial one. No significant differences were found in isoprostane concentration in stimulated saliva before and after prosthetic treatment.

CONCLUSION: Prosthetic dental treatment leads to decrease in oral oxidative stress.

Introduction

Free radicals – reactive oxygen species (ROS) and reactive nitrogen species (RNS) take part in the normal metabolic reactions, in signaling pathways and defense mechanisms of the human body. In healthy organism, there is balance between their production and neutralization. When this equilibrium is disturbed, a state of oxidative stress occurs. Because of their high reactivity ROS and RNS may damage different types of biomolecules – lipides, proteins, and nucleus acids [1], [2]. Lipid peroxidation, as a result of free radical damage, may lead to disturbance of structure and function of cell membranes, cell apoptosis, autophagy and formation of potentially mutagenic and carcinogenic products [3]. At state of long-term chronic oxidative stress, ROS contribute to the development of numerous pathogenetic mechanisms and to different stages of carcinogenesis [4].

According to Avezov *et al*., 2015, there are several factors that may induce oral oxidative stress when acting either separately or together – inflammatory diseases, dental materials, cigarette smoking, food ingredients, and alcohol intake [5].

In the oral cavity, the disturbance of balance between prooxidant and antioxidant mechanisms and the development of oxidative stress are considered as major factors in pathogenesis of oral mucosal diseases [6]. Bacterial invasion at periodontal diseases induces local immune response which limits the spread of microorganisms. Increased formation of free radicals takes part in the defense against viral and bacterial agents [7]. However, oxidative stress as a result of oral inflammation may cause potential damage of DNA and apoptosis [8]. High levels of plasma oxidative stress markers are found in patients with aggressive periodontitis [9]. Recently, studies found a correlation between oxidative stress caused by systemic disorders (type II diabetes and obesity) and the progression of periodontal pathology [10], [11], [12].

Dental materials such as resin composites, glass ionomer cements, root canal filling materials, and dental alloys may disturb the local balance between formation and neutralization of free radicals [13].

Prosthetic treatment requires fabrication of dental restorations from foreign to the body materials with different chemical, mechanical, and biological properties which must provide longevity, esthetics, and safe use. Pure metals, noble, and base alloys are used for production of inlays, onlays, splints, implants, and post-and-core restorations. In combination with other materials such as ceramics, laboratory composites, and acrylic resins, they are used for prosthetic rehabilitation in the oral cavity. With the development and the constantly increasing use of CAD/CAM technologies the number of full ceramic restorations increases, but metal ceramic restorations are still preferred in most clinical cases because of their excellent final mechanical properties, good esthetics, long-term use, and comparatively acceptable price. The most important feature of a dental alloy for its biological safety is its tendency to corrosion. Corrosion is the process of deterioration of the metal objects under the influence of agents of the surrounding environment [14]. After placing a metal object in an electrolyte, the surface starts to ionize, the metal starts to dissolve, and metal ion emission is observed. During mastication because of mechanical loading and grinding tribocorrosion appears and increase of metal ion release may be observed [15]. Metal ions released from the surface come in contact with the surrounding tissues and can be spread out into the body through the gastrointestinal system [16].

Metal ions emitted from medical and dental biomaterials can influence the equilibrium between prooxidant and antioxidant activity in the body and may induce oxidative stress leading to chronic inflammations and numerous other diseases such as allergies, oral lesions, and changes in perception of taste [17]. The toxicity of cobalt and chromium ions depends on their valence. Entered the body, they undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in the plasma and mitochondria [18]. Chen *et al*., 2015, confirmed that Cr^{6+} has greater permeability through the cell membrane compared to Cr^{3+}. The change in the valence as a result of intracellular reduction processes leads to ROS formation which may cause oxidative stress and further affect DNA [19]. Studies of Battaglia *et al*., 2009, showed that Cobalt ions (Co^{2+}) may cause apoptosis in liver cells because of induced oxidative stress in mitochondria [20]. Permenter *et al*., 2013, found that cobalt ions cause increased oxidative stress level and change in gene and protein expression in different cell lines [21]. Copper ions (Co^{2+}) may lead to change in osteoclastic activity and increased formation of ROS in osteoclasts [22]. Spalj *et al*., 2012, found that stainless steel orthodontic wires cause oxidative stress in L929 fibroblasts in mice [23]. According to Bandeira *et al*., 2020, stainless steel induces cytotoxic effects in gingival fibroblasts and increased expression of antioxidant genes – a sign of oxidative stress [24].

Products of interaction between ROS and different biomolecules may be used for evaluation of the oxidative stress level in the body. Unlike prostaglandins (PG), which are formed from free arachidonic acid under the action of cyclooxygenases (COX), isoprostanes are formed because of nonenzymatic lipid peroxidation of arachidonic acid and its esters, which are part of cell membranes. The release of isoprostanes from membrane structures happens under the action of phospholipases and platelet-activating factor acetyl hydrolase (PAF-AH) [25]. The use of isoprostanes as a marker of oxidative stress level has two advantages – their low chemical reactivity and presence in all biological fluids [26]. Furthermore, their local concentration may be used for assessment of the specific body system or area [27]. Increased level of 8-isoprostaglandin F2α in blood plasma is found at patients with oral leukoplakia and in patients with periodontitis [28], [29]. In patients with chronic periodontitis increased concentration of 8-isoPGF2α is detected in saliva [30].

Concerns about biological impact of the ion emission from the alloys used in dentistry guided us in defining the aim of the study. The research hypothesis was that the corrosive changes and the metal ion emission from the base dental alloy used for production of the prosthetic metal ceramic restoration would cause an increase in the oral oxidative stress level.

The aim of this study was to evaluate the influence of the prosthetic dental treatment with metal ceramic restorations on the level of oxidative stress in the oral cavity.

Materials and Methods

Metal ceramic crowns with CoCr copings fabricated by direct metal laser sintering (DMLS) were produced for 35 patients using CAD/CAM technology. After taking digital impressions with Trios intraoral scanner (3Shape, Denmark), the metal copings were designed, and the files were sent to the 3D printer EOS M100, (EOS, Germany), present at the CAD/CAM Center of FDM-Plovdiv. CoCr dental alloy EOS CobaltChrome SP2 (EOS, Germany) was used to produce the metal copings. The composition of the alloy according to the producer was: Co: 63.8 wt-%; Cr: 24.7 wt-%; Mo: 5.1 wt-%; W: 5.4 wt-%; Si: 1.0 wt-%; Fe: max. 0.50 wt-%; Mn: max. 0.10 wt-%; free of Ni, Be, Cd, and Pb according to ISO 22674. 3D printed resin models were used for finishing the PFM restorations. The crowns were fixed with resin-modified glass ionomer cement Ketac Cem Plus (3M ESPE, USA).

All patients included in the study have signed informed consent. All procedures performed in the studies were in accordance with the standards of the Institutional Ethical Committee of Medical University of Plovdiv, Bulgaria (Decision № C – 03-2/10.04.2020) and with the Association Declaration of Helsinki 1964.

Participants had to meet the following criteria: non-smokers at the age between 18 and 65, without acute or chronic diseases at the beginning of the study.

Patients were instructed not to take any food or drinks except water before the dental visit. Non-stimulated and stimulated saliva samples were taken from the patients before the beginning of prosthetic treatment, 2 h, and 7 days after placing the metal ceramic restorations in the oral cavity. Samples were gathered in the dental office by spitting in low density polyethylene containers (LDPE) in the interval between 9.00 a.m. and 12.00 a.m. to avoid circadian variations, without exposure to any visual, taste or aromatic stimuli. The patients were in a seated position, with the head slightly inclined forward. After rinsing the mouth with distilled water, the patients spat the saliva gathered at the bottom of the oral cavity for 15–20 min. After gathering 15 ml of non-stimulated saliva, 5 ml were placed in a centrifugal tube for detection of isoprostane 8-isoPGF2-alpha. Stimulated saliva samples were taken after placing 2% citric acid over the tongue (100 μL each 30 s) for 5 min. The samples were immediately frozen at –20°C and later transferred to the Research Institute at Medical University of Plovdiv for storage at –70°C. For evaluation of oxidative stress level in the oral cavity concentration of 8-isoPGF2-alpha was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) before, 2 h, and 7 days after crown cementation. The concentration of 8-isoPGF2-alpha was measured using system UHPLC Thermo Dionex Ultimate 3000 with mass detector Thermo Quantum Access Max (Thermo Fisher Scientific, MA, USA).

For statistical processing SPSS statistical package, version 19.0 was used. Non-parametric Wilcoxon signed-rank test and Mann–Whitney test were applied. Level of significance was $p \leq 0.05$.

Results

The descriptive analysis of concentration of 8-isoPGF2-alpha is given at Table 1.

Table 1: Characteristics of 8-isoPGF2-alpha concentration in non-stimulated (NS) and stimulated saliva (SS)

Isoprostanes	Min	Max	Mean ± SD	Median
NS before	0.00	82.79	15.74 ± 23.57	4.84
NS 2 h	0.11	77.93	7.54 ± 14.17	2.85
NS 7 days	0.04	94.54	9.20 ± 19.83	1.48
SS before	0.00	7.08	1.64 ± 1.78	0.78
SS 2 h	0.00	18.51	2.14 ± 3.38	1.06
SS 7 days	0.02	157.21	7.02 ± 27.16	1.56

The concentration of isoprostane 8-isoPGF2-alpha in non-stimulated saliva was lower 2 h after fixing the PFM crown compared to the initial level and statistically significant difference was observed. 7 days after placement of the restoration the level of 8-isoPGF2-alpha slightly increased but no statistical significance was found. On the 7th day, the concentration of isoprostanes remained significantly lower than the initial one. The detected mean values of isoprostane concentration in stimulated saliva samples slightly increased after the initial measurement but no statistically significant differences were found before, 2 h, and 7 days after prosthetic treatment (Table 2 and Figure 1).

Table 2: Comparison of 8-isoPGF2-alpha concentration before and after prosthetic treatment in non-stimulated (NS) and stimulated saliva (SS)

Isoprostane concentration comparison	NS		SS	
	Paired differences in Mean ± SD	p-value	Paired differences in Mean ± SD	p-value
Before treatment– 2nd h	7.87 ± 15.88	0.022	–0.50 ± 2.30	0.525
2nd h – 7th day	–1.27 ± 10.83	0.556	–5.53 ± 27.78	0.543
Before treatment – 7th day	7.00 ± 13.79	0.017	–5.10 ± 27.85	0.291

Level of significance: $p \leq 0.05$.

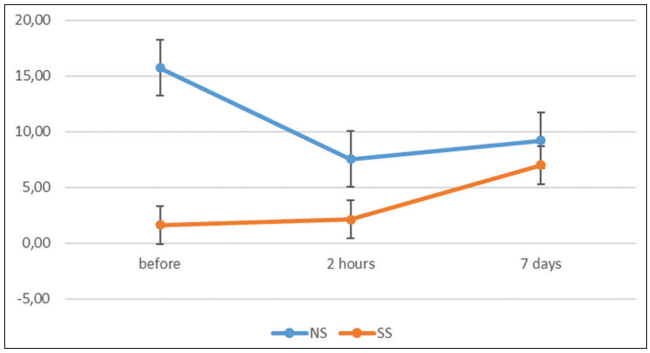

Figure 1: Changes in concentration of 8-isoPGF2-alpha concentration in non-stimulated (NS) and stimulated saliva (SS)

At the initial measurement and the measurement on the 2nd h after fixing the metal ceramic crown, the isoprostane concentration in non-stimulated saliva was significantly higher than the one in stimulated saliva samples. There were no statistically significant differences in isoprostane level from stimulated and non-stimulated saliva on the 7th day (Figure 2).

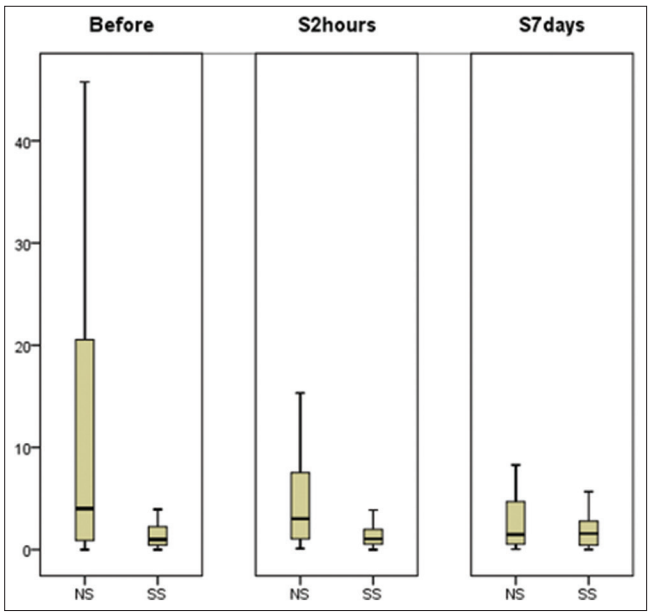

Figure 2: Comparison of 8-isoPGF2-alpha concentration in non-stimulated (NS) and stimulated saliva (SS)

Discussion

All laboratory analyses require the presence of a sample size larger than the minimally defined one in case reanalysis is needed. The necessary saliva sample size for 8-isoPGF2-alpha detection was determined using saliva pull during the LC-MS/MS method development. A minimal sample size of 1 ml was defined. Regarding the amount of saliva needed and the time needed for sample collection, a sample size of 5 ml was chosen [31].

Isoprostanes play an important role in signaling cell pathway and possess numerous other functions – they are a potent vasoconstrictor, may induce endothelin release and proliferation of vascular smooth muscle cells [32]. Formation of 8-isoPGF2-alpha is a result of interaction of free radicals with lipids present in cell membranes. Isoprostane concentration may indicate possibility of cell damage caused by ROS and RNS. Oxidative stress in the oral cavity may be induced by some dental materials and because of inflammatory diseases of the mucogingival and periodontal tissues [5].

The results of our study do not correspond to the findings of Kovač *et al*., 2020, according to which CoCr alloys induce increase in oxidative stress level [33]. Our study confirms the conclusions of McGinley *et al*., 2013, which found that dental CoCr alloys do not elicit adverse oxidative stress [34].

The correlation between inflammatory oral diseases and the level of oxidative stress is studied by numerous researchers [35], [36]. It may be assumed that the elimination or decreasing of the level of inflammation would lead to decrease in oxidative stress level. This hypothesis is supported by the studies of Ekuni *et al*., 2008, and Kamodyová *et al*., 2013, according to which the improved oral hygiene and better tooth brushing leads to elimination of the dental plaque, containing ROS producing bacteria, and to increased level of crevicular fluid containing antioxidant components [37], [38]. The decrease in oxidative stress level in the oral cavity may be due to improved oral hygiene measures during the period of prosthetic treatment, requiring several visits in the dental office, which may have led to decrease in inflammatory processes in the soft tissues.

From the statistically insignificant change of 8-isoPGF2-alpha concentration in stimulated saliva, it may be assumed that the locally applied measures from the dentist (prosthetic treatment and dental materials) and from the patients (hygienic procedures) do not influence the level of oxidative stress in salivary glands, that is, in areas which are not in direct contact with the oral cavity. Saliva, produced in the salivary acini, cannot be considered as analog of blood plasma. Therefore, from saliva analysis it cannot be concluded whether local oral changes affect the general oxidative stress level in the body. This statement is supported by the studies of Sazanov *et al*., 2017, and Lee *et al*., 2018 [39], [40]. However, Zugla *et al*., 2019, found a correlation between levels of oxidative stress markers in saliva and plasma and concluded that saliva may be used as a medium for assessing the level of oxidative stress in the human body [41].

Conclusion

Metal ceramic restorations with copings of CoCr dental alloys produced by DMLS using CAD/CAM technology do not induce adverse changes in oral oxidative stress level. Prosthetic dental treatment leads to decrease in oxidative stress in the oral cavity.

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