NITRIC OXIDE – MARKER OF OXIDATIVE STRESS IN DIFFERENT TYPES OF FIXED DENTAL RESTAURATIONS

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ABSTRACT

The aim of the present study is to evaluate the level of oxidative stress by quantifying salivary Nitric Oxide in patients with three types of fixed prosthetic: porcelain fused to metal crown; all ceramic; zirconium ceramic. **Material and Method.** The study was performed on 50 subjects, divided in three groups for each type of fixed dental restauration. Nitric oxide was evaluated by the immunochromatographic method (Nitric Oxide Saliva Test Strips, Berkeley, USA) before and after the permanent cementation of the three different fixed prosthetic. **Results.**The highest values of Nitric Oxide were quantified in the patients group with porcelain fused to metal crown, after seven days and after six months with statistically significant differences between this values *(p=0.0006);* there are less statistical significance regarding the values of the same determinations in patients with all ceramics *(p=0.02)* and no statistically differences regarding the values of these determinations in patients with zirconium ceramic *(p=0.32).* Also, there are statistically significant differences regarding Nitric Oxide values in patients with porcelain fused to metal crown and those with all-ceramic *(p=0.0001)* and zirconium-ceramic *(p=0.0007)* after six months demonstrating that the highest level of oxidative stress is produced by metal-ceramic prosthetic materials. **Conclusions.** The evaluation of the Nitric Oxide level can be used to assess the intensity of oxidative stress in the oral cavity of patients rehabilitated orally with prosthetic works made of different materials.

Key words: nitric oxide, oxidative stress, oral rehabilitation

INTRODUCTION

Energy production at the cellular level is one of the main links that ensure life; the complete oxidation of carbohydrates releases together with energy and a varied range of free radicals also called reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. If they are produced in moderate quantities, they are useful for normal cellular physiology, being in a perfect balance with the antioxidant systems; in large quantities, however, this balance disappears, oxidative stress (SO) sets in, free radicals affecting the cells' own structures [1,2]. The transformation of free radicals into inactive molecules is possible through the action of enzymatic antioxidant substances (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)), and non-enzymatic glutathione, albumins, ascorbic acid (AS), uric acid (AC) and melatonin [1, 2, 3].

Oxidative stress is defined as an imbalance between ROS, RNS and antioxidant systems; in this situation, free radicals destroy the lipids in the cell structure through the oxidation process, damage the proteins through peroxidation and seriously modify the DNA by breaking the phosphodiester bonds of the nitrogenous bases [2, 3].

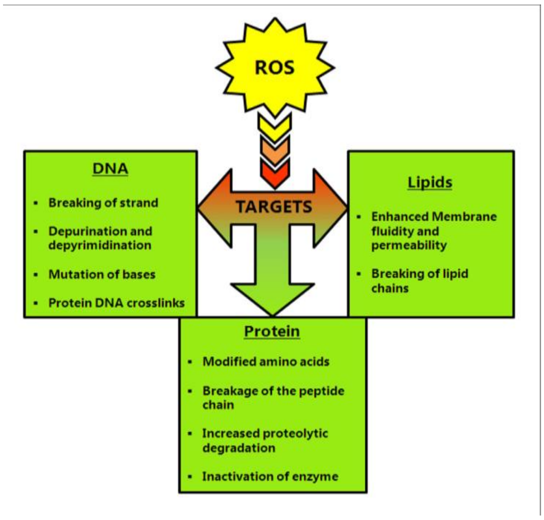

Figure. 1 Various targets of ROS [4]

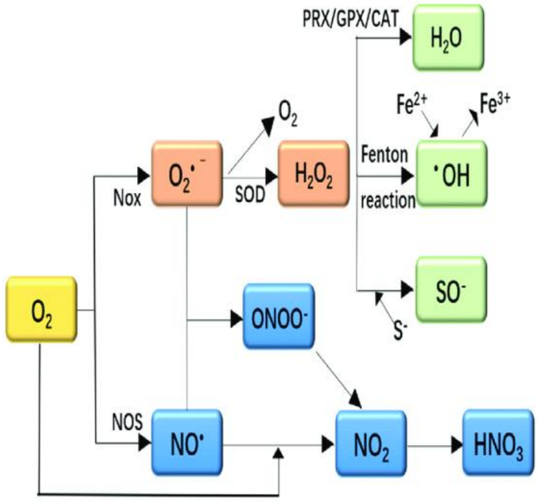

Figure 2. ROS and oxidative stress [10]

All these cellular changes are identified as links in the emergence of certain diseases, studies presented in the specialized literature showing that oxidative stress is involved in the pathophysiology of over 200 diseases, including periodontitis, diabetes, atherosclerosis, cardiovascular diseases, neurological diseases, different types of cancer, including aging processes [5, 6]. Through the oxidation of L-arginine, nitric oxide (NO) and L-citrulline are produced as a secondary product; NO is a gas with versatile action, participating in low concentrations in physiological processes that support cellular homeostasis; intervenes in the regulation of bone tissue metabolism, is mainly synthesized in macrophages and endothelial cells and has an important role in the regulation of vascular tone. It also reduces the adhesion of neutrophilic polymorphonuclear leukocytes and blocks the production of inflammation at the level of the vascular walls [7]. The presence of bacteria [8], through the properties of the wall polysaccharide, stimulates the synthesis of large amounts of NO; the influx of antigens (bacterial, viral, parasitic cancer) induces the synthesis of pro-inflammatory mediators such as IL-1 beta, IL-1, IL-6, TNF-alpha which will stimulate the synthesis of large amounts of NO in macrophages [9] .

The ions of metal alloys used to make dental prostheses can induce oxidative stress with possible pathological changes in the oral cavity. Starting from the hypothesis that metal ions released from these alloys can induce oxidative stress, the aim of the present study is to evaluate the level of oxidative stress by quantifying salivary NO in patients with different types of fixed prosthetic works.

MATERIAL AND METHODS

1. *Study group and criteria for patient selection*

The study group consisted of 230 subjects that were examined clinical and radiological in the Department of Oral Rehabilitation, Faculty of Dental Medicine, Ovidius University from Constanta.

Among them, we have selected 50 subjects (20.17%), subjects that passed the exclusion criteria such as: smoking, generally traumatic oclusion, systemic disease, pregnancy, treatment with antibiotics and pain killer in last 6 month.

Each patient was clinically evaluated using Probing depth (PD), Gingival index (GI), Plaque index (PI), and finnaly we formed three study groups according to the fixed resturation that were used, as follows: group 1 - porcelan fused to metal, 10 subjects; group 2 - all ceramic, 15 subjects and group

3 - zirconium ceramic, 25 subjects.
Before the fixation of the restauration the teeth were prepared and all of the teeth involved in this study were endodontically treated. We cimented the resturation after seven days, so all of this process would not alter our study pattern.
Before we cement the resturation the first quantification of NO was performed and then, after six months the second determination of NO.

2. Nitric Oxide qantification

It was done by the immunochromatographic method following the instructions stipulated by the manufacturer (Nitric Oxide Saliva Test Strips, Berkeley, USA), as follows: the strip was placed on the tongue for 10 seconds, then it was folded and kept in contact for another 10 seconds.
The obtained result was expressed semi-quantitatively, compared with the colorimetric map available on the box, with 5 divisions corresponding to the following quantities: 20µM, 110µM, 220µM, 435µM, 870µM.

Figure. 3 Nitric Oxide Saliva Test, Berkeley, USA

3. Statistical analyses

Statistical analysis was performed using SPSS 14.0 for Windows and MedCalc 11.3.0. The statistical differences between the NO levels from the three study groups were analyzed by using Student's t-test, statistically significance of the results was defined for $p<0.05$ (two tail).

4. Ethical permission

All participants were informed about the study; ethical permission to conduct this study was given by the Professional Ethical Committee of Ovidius University from Constanta, in order to respect the ethical principles for medical research involving human subject, given by the world Medical Association Declaration of Helsinki; inclusion in the study group was based on the written consent obtained from all the participants.

RESULTS

The results regarding the mean and standard deviation of the quantified NO values before and after the definitive fixation of the three types of prosthetic restaurations are presented in table 1.

Table 1. Means and standard deviations of the NO before and after the cementation of the three types of fixed restaurations

	Time of evaluation	Mean value and Std. Deviation
Group 1(10 subjects) - PORCELAIN FUSED TO METAL	NO before	47±43.4
	NO after	468±43
Group 2 (15 subjects) – ALL CERAMIC	NO before	32±31.6
	NO after	72±82.8
Group 3 (25 subjects) - ZIRCONIUM CERAMIC	NO before	41,6±39.2
	NO after	46±51.4

The statistical processing of the obtained results shows that there are differences with high statistical significance regarding the NO values between the first and second determination in patients with porcelain fused to metal crown *(p=0.0006)* and differences with less statistical significance regarding the values of the same determinations, in patients with all ceramics *(p=0. 02)*; also, there are no statistically significant differences regarding the values of these determinations in patients with zirconium ceramic *(p=0.32)*, as seen in table 2.

Table 2. Statistical analyses of NO levels between before and after the cementation of the three types of fixed restaurations

NO	Statistical significance *p<0.05; t-test*
Group 1 before/after	*p=0.0006*
Group 2 before/after	*p=0.02*
Group 3 before/after	*p=0.32*

In the same time, statistical processing of the results obtained show highly significant statistical differences in NO values quantified after the mounting of the restoration between porcelain fused to metal (Group 1 subjects) and all ceramic (Group 2 subjects) (*p=0.0001*) and between porcelain fused to metal crown (Group 1 subjects) and zirconium ceramic (Group 3 subjects) *(p=0.0007*). There is no statistical differences in NO values between all ceramic (Group 2 subjects) and zirconium ceramic (Group 3 subjects) *(p=0.22*), as shown in table 3.

Table 3. Statistical analyses of NO between the three study groups after six months.

NO	Statistical significance *p<0.05; t-test*
Group 1/ Group 2	*p=0.0001*
Group 1/ Group 3	*p=0.0007*
Group 2/ Group 3	*p=0.22*

DISCUSSION

The importance of studying NO as a marker of oxidative stress is demonstrated by numerous studies that quantify or monitor NO dynamics in the context of diseases occurring at a distance from the oral cavity (cardiovascular) [11], but also in the oral cavity in various forms of periodontitis [12, 13, 14] and in patients with orthodontic appliance [15] and oral cancer [16]. The development of knowledge in dental medicine also had an important impact in oral rehabilitation, through the use of new materials and technologies for obtaining dental prostheses, starting from metal-ceramic combinations, all-ceramic and zirconium ceramic [17, 18, 19, 20]. Although the development of CAD/CAM technologies has allowed the number of all-ceramic works to increase, metal-ceramic restorations are still preferred by patients due to their good long-term mechanical resistance and affordable price, although there is a great inconvenience related to the possibility of releasing some variable quantities of metal ions that induces oxidative stress in the oral cavity [21, 22]. Unlike metal ceramic, zirconium has proven to be, for now, a clearly superior material due to its biological, mechanical and optical qualities, non-toxic, non-mutating, non-carcinogenic, as shown by studies on human fibroblasts [23].

The results of the present study show that the highest level of oxidative stress is produced in the oral cavity at patients with metal-ceramic prostheses between the values quantified 7 days, respectively 6 months after fixing the prosthetic works. Also, there are statistically significant differences between the NO values quantified in patients with porcelain fused to metal crown and those with all-ceramic and zirconium ceramic, demonstrating that the level of oxidative stress produced by this materials is high; there are no statistically significant differences regarding the NO level between patients with all-ceramic and zirconium-ceramic restorations after each of the two determinations , demonstrating that these materials do not induce an important

oxidative stress in the oral cavity. The highest values of NO when determined after 7 days in patients with metal-ceramic prostheses demonstrate that NO mediates the early phases of the inflammatory process, which allows us to state that it is a sensitive indicator of inflammation, with perspectives to be used as a marker of assessment of other inflammatory processes in the oral cavity.
There are few studies regarding the quantification of NO as a possible marker for evaluating oxidative stress in the oral cavity, studies that use other quantification methods; thus Magdu W. at all indirectly evaluates NO production, spectrophotometrically measuring nitrates and nitrites, in different groups of pregnant women, demonstrating that oxidative stress increases with gestational age [24].
Given that people pay more and more attention to oral health, the promotion of innovative research on the optimization of oral rehabilitation methods is very important. From these considerations, we appreciate that deepening the knowledge of the materials used to make dental prostheses, from the point of view of oxidative stress, is still a priority in the field of dental medicine.

CONCLUSIONS

The evaluation of the NO level can be used to assess the intensity of oxidative stress in the oral cavity of patients orally rehabilitated with prostheses made of different materials.

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