

OXIDATIVE STRESS, NITRIC OXIDE AND ITS RELATIONSHIP WITH PERI-IMPLANTITIS

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Abstract:

Introduction The study aims to evaluate oxidative stress by quantifying nitric oxide (NO), as well as identifying the possible correlation between NO and clinical parameters in peri-implantitis in order to optimize the diagnosis and monitoring of this condition. **Material and method** The study was carried out on three groups of patients with dental implants, selected after applying the established inclusion and exclusion criteria, as follows: patients with a favorable evolution, patients with mucositis and patients with peri-implantitis. The evaluation of salivary (NO) was done after 4 and 12 weeks after the insertion of dental implants using NO strips (Berkeley test strip, USA). **Results** of the present study show that there are statistically significant differences regarding salivary NO values after both moments of quantification between the groups of patients with favorable evolution and patients with peri-implantitis ($p=0.0001$; 0.0003); there are no statistically significant differences between the groups of patients with mucositis and peri-implantitis for both moments of salivary NO determination. Also, there is a correlation between NO values and clinical parameters, respectively the depth of the peri-implant groove) for patients with mucositis ($r=0.676$; $r=0.725$) and peri-implantitis ($r=0.650$; $r=0.844$) for both determination moments. **Conclusions** of the study are that NO can be considered a marker for estimating the inflammatory process in the context of peri-implantitis. The facility with which NO is determined in saliva, recommends that the test be used in the dental office as a biomarker in the diagnosis and monitoring of peri-implantitis.

Keywords: nitric oxide, pei-implantitis, rapid strip testing

Introduction

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) together with free nitrogen radicals (RNS) and their neutralization mechanisms, respectively antioxidant systems [1]. Reactive

oxygen and nitrogen species are physiologically produced, in small quantities, following normal cellular metabolic reactions; in this context, NO is a signaling molecule involved in numerous metabolic pathways [1,2]. The state of health is defined by a perfect balance between the production of

these radicals and the antioxidant systems, the results of the imbalance between these elements being: lipid peroxidation, protein oxidation and serious changes in DNA and finally, cell death by apoptosis [1,2,3].

Nitric oxide-NO was discovered in 1722 by Joseph Priestley as a colorless, extremely unstable gas; after 1980 the involvement of NO as an endothelial relaxation factor was studied, this discovery increasing the interest of research related to this gas. In-depth studies on this gas have shown that it is in fact a nitrogen free radical, and highly reactive, resulting from the oxidation process of L-arginine, through the catalytic action of nitric oxide synthase (NOS), thus resulting in NO and L-citrulline, as seen in Figure 1.

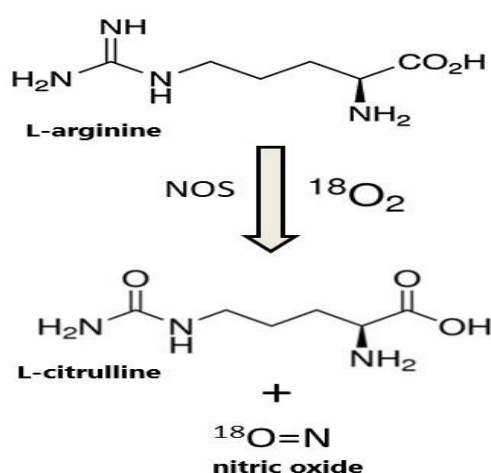


Figure 1. Biosynthesis of nitric oxide from L-arginine- NO synthase (NOS) catalyzes the oxidation of L-arginine to NO and L-citrulline by using molecular oxygen [4]

Another important source of NO in the human body is represented by nitrate-NO₃- from food which is initially metabolized by the bacteria in dental plaque into

nitrites-NO₂- and then transformed into NO. Three distinct NO isoform families are known, whose coding is done in different gene groups; in moderate concentrations, these types of NO contribute to the body's homeostasis, having beneficial effects, as follows:

- neuronal-NOS 1, (n NOS), with effect in regulating synaptic transmission;
- inducible-NOS 2, (i NOS), produced in those involved in immunity, regulation of immune reactions, with important antibacterial and antitumor properties
- endothelial-NOS 3 (e NOS), identified in endothelial cells and with a role in vascular regulation, respectively in the process of vasodilatation with a chemotactic effect and influx of neutrophilic polymorphonuclear leukocytes [4].

In low concentrations, NO has beneficial properties, as presented above, the anti-inflammatory and antioxidant properties being achieved by eliminating the superoxide anion. The anti-inflammatory properties of NO are mainly due to its ability to inhibit the production of pro-inflammatory cytokines such as IL-1 β and TNF- α . In the oral cavity, a large amount of iNOS is produced in response to bacterial endotoxins; it can act as a non-specific antibacterial defense system or by increasing the efficiency of macrophage functions. In high concentrations, NO forms a strong oxidant-peroxynitrite that contributes to the triggering of the inflammatory process, generates cytotoxicity by damaging membrane phospholipids, seriously disrupting

mitochondrial functions and cell apoptosis, as shown in Figure 2.

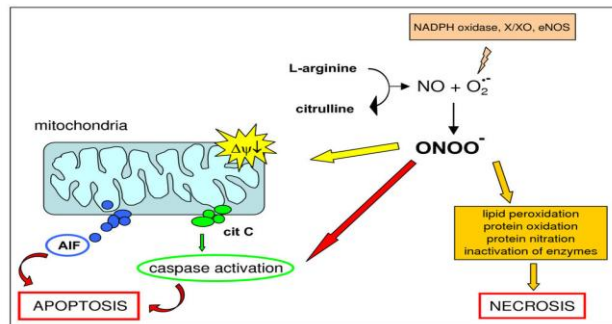


Figure 2. Reaction of peroxynitrite leading to either apoptotic or necrotic cell death [5]

The binding of oxygen molecules leads to the stabilization of NO, resulting in stable final products, namely nitrate NO₃⁻ and nitrite NO₂⁻ [4,6,7]. It is appreciated that the oxidative stress resulting from imbalances between the pro-oxidant and antioxidant mechanisms is a major factor in the etiopathogenesis of many diseases, including the production of periodontal disease [8]. In this vein, in the context of periodontal disease, there are numerous studies in the specialized literature that demonstrate that through the presence of bacterial antigens, a process of stimulation of polymorphonuclear leukocytes and the release of a high level of oxygen and nitrogen free radicals takes place, especially NOS, with the occurrence of oxidative lesions and subsequently, the destruction of periodontal ligaments and alveolar bone [9,10,11]. A challenge in the practice of dental medicine is represented by peri-implantitis, a complication defined by an inflammatory process that affects the soft and hard peri-implant tissues with a high risk of implant loss [12,13,14,15]. Given that

titanium and its alloys are the basic materials used in the manufacture of implants, it is appreciated that the bacterial colonization of the implant, together with the titanium ions identified in the peri-implant tissues, can be the main elements that trigger oxidative stress with the stimulation of the synthesis of cytokines IL1-beta type and the eventual appearance of peri-implantitis [11,12]. The common elements related to the etiopathogenesis of peri-implant and periodontitis regarding pathogenic bacterial species [15], the mechanism of action of pro-inflammatory cytokines within the inflammatory process in the two conditions, are demonstrated by numerous studies following the evolution of patients with peri-implantitis and periodontitis in the long term short [17,18], but also in the long term [19,20]. However, the role of oxidative stress is demonstrated only in periodontitis, but in peri-implantitis this subject remains insufficiently known and largely unclear. Given these similarities between periodontitis and peri-implantitis regarding the etiopathogenesis, the hypothesis from which the present study starts is that in peri-implantitis oxidative stress can be an important link that contributes to the appearance of this condition. The present study aims to quantify nitric oxide in the context of oxidative stress, as well as evaluate the possible correlation between the nitric oxide level and clinical parameters in order to optimize the diagnosis of peri-implantitis.

Materials and Methods

2.1 Study group

The study group consisted of 310 patients (women-190, 61.29%; men-120, 38.71%), presented in the private office of dentistry and in the Department of Implantology of the Faculty of Dentistry, "Ovidius" University of Constanta, patients who had 351 dental implants inserted between february and may 2025. One month after dental implant insertion the patients were evaluated clinically and paraclinically using clinical parameters according to the established protocols [15] and it was found that 286 patients (91.60%) had a favorable evolution after dental implant insertion and 24 (8.40%) which had an unfavorable evolution. From the group of patients with favorable evolution, we randomly selected 19 patients who constituted the control group; to these we added the 24 patients with unfavorable evolution; following the clinically and paraclinically examination, the patients in the study group were classified into 3 categories in the following order: 19 patients with favorable evolution of the dental implant, 15 mucositis patients and 9 peri-implantitis patients. After each clinical and radiological evaluation, T1-at 4 weeks; T2-at 12 weeks, patients from all three study groups performed oxid nitric quantification using Nitric Oxide Saliva Test Strips (Berkeley, CA, USA) .

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
✓ More than 25 years	■ History of periodontitis
✓ Least three	■ Sistemic-diseases
	■ Pregnancy

prothetic implants	■ Smoking ■ Antibiotic therapy the last 6 months ■ Antiinflammatory therapy the last 6 months ■ Poor oral hygiene
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2. 2 Nitric Oxide detection and quantification by semi-quantitative method

Nitric Oxide detection was performed used Nitric Oxide Saliva Test Strips (Berkeley, CA, USA), test is based on the clinically validated Griess reagent reaction; the test performs the indirect quantification of through nitrite as a stable precursors of NO. The strip was placed on the tongue for 5 seconds, folded the strip over and press the two sides together in contact for another 10 seconds. The reaction is based on the turn of a marker for NO, so that a color reaction was generated-from white to pink with a directly proportional intensity to the level of nitrites present in saliva. The results obtained was compared with the colorimetric map available on the box for the semi-quantitative assessment depleted, low, threshold, target, high, corresponding to the following levels of nitric oxide expressed in $\mu\text{M/L}$: 20,110, 220, 435, 870 $\mu\text{mol/L}$ (Figure 3). All determinaton were performed in duplicat, without differences between them.



Figure 3. Nitric Oxide Saliva Test Strips (Berkeley, CA, USA)

2. 3. Statistical Analyses

The results were statistically analysed using SPSS 14.0 for Windows and MedCalc Software 11.3.0. The statistically differences between the levels NO 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). We used Pearson correlation test (r-correlation coefficient) to identify correlation between the analysed parameters; $p\text{-value} < 0.05$ was considered statistically significant.

2.4 Ethical permission

The study was conducted in accordance with the ethical principles of the Helsinki Declaration; the study was approved by the Bio-Ethics Committee of "Ovidius" University, Constantza, Romania. Written consent of each patients was obtained after the procedure was explained.

Results and Discussions

The average value of nitric oxide is the highest in the group of patients with peri-implantitis, followed by those with mucositis; the lowest values were recorded in the control group, as seen in Table 2.

	Time of evaluation	Mean value Std. Deviation ($\mu\text{mol/L}$)	Time of evaluation	Mean value Std. Deviation ($\mu\text{mol/L}$)
control	4 weeks (T1)	190.66 $\pm$ 50.3	12 weeks (T2)	158 $\pm$ 83.9
mucositis	4 weeks (T1)	355.78 $\pm$ 106.5	12 weeks (T2)	178.94 $\pm$ 103.6
peri-implantitis	4 weeks (T1)	435.55 $\pm$ 187.6	12 weeks (T2)	279.44 $\pm$ 243.2

Table 2. Mean and standard deviation of nitric oxide for the study groups

The obtained results show that there are statistically significant differences between the three study groups, both in the evaluation after 4 weeks and in the evaluation made after 12 weeks, with the exception of the mucositis/peri-implantitis groups ($p > 0.05$) as can be seen in Table 3.

Table 3. Statistical analyses of nitric oxide between the three study groups after 4 and 12 weeks

Also, there are statistically significant differences regarding the levels of NO quantified after 4 and 12 weeks within each of the three study groups (Table 4).

NitricOxide 4 weeks/12 weeks	control	mucositi s	peri-impla ntitis
<i>t-test; Statistical significance $p<0.05$</i>	$p=0.019$	$p<0.0001$	$p=0.008$

Table 4. Statistical analyses of nitric oxide between the levels of each study groups after 4 and 12 weeks

The obtained results show that the levels of nitric oxide correlate with the depth of the peri-plantar groove, both at the first evaluation (Table 5) and at the second evaluation of the three groups of patients (Table 6), the highest level of correlation among the two parameters being recorded in the case of patients with mucositis ($r=0.724$) and peri-implantitis ($r=0.844$).

NitricOxid e/Probing depht (Pearson corellation)	control -4 weeks	mucositis -4 weeks	peri-implantitis -4 weeks
<i>p</i>	0.211	0.001	0.05
<i>r</i>	0.342	0.676	0.650

Table 5. Corellation between nitric oxide levels and probing depht after 4 weeks

Nitric Oxide	Statistical significance $p<0.05$; <i>t-test-4 weeks (T1)</i>	Statistical significance $p<0.05$; <i>t-test-12 weeks (T2)</i>
control/ mucositis	$p<0.0001$	$p=0.053$
control/peri-implantitis	$p=0.0001$	$p=0.0003$
mucositis/peri-implantitis	$p=0.16$	$p=0.133$

NitricOxide/Probing depht (Pearson corellation)	control-1 2 weeks	mucositis -12 weeks	peri-impl antitis -12 weeks
<i>p</i>	0.57	0.0004	0.004
<i>r</i>	0.156	0.724	0.844

Table 6. Corellation between nitric oxide levels and probing depht after 12 weeks

The development of dental implantology represented a crucial moment in the practice of dental medicine, with a particular impact on the oral rehabilitation of partially or totally edentulous patients [12,13], the restoration of facial aesthetics and the recovery of masticatory function being among the most important advantages gained by patients after the insertion dental implants [21]. Also, recent reports (2023) WHO show that in a top 300 diseases listed by WHO, diseases of the oral cavity occupy the first place with an impressive figure - 3.5 billion people worldwide suffering from various diseases of the oral cavity, among which approximately 510 million are edentulous patients. At the same time, the trend of population aging worldwide has become a certainty and it is estimated that in 2019 the population over 65 years old was around 700 million, and by

2050 it will reach 1.5 billion [22,23]. In these conditions, the expectations related to the increase in the quality of the implants, the insertion procedures used, but also the discovery of ways to prevent possible complications are very high. But, with all the progress registered in implantology, peri-implantitis remains a major cause of failure, a certainty demonstrated by statistical studies that show a prevalence of this condition of approximately 19% of all patients in whom implants were inserted and 12% of the total number of implants inserted globally [24]. Currently, titanium and its alloys are used for the manufacture of dental implants given its biocompatibility, but also for the special mechanical resistance demonstrated over time; however, the titanium ions and titanium dioxide nanoparticles identified in the peri-implant tissues can initiate its pathological changes, but can also initiate bone resorption processes [25]. In vivo studies show that the oxidative stress generated by free radicals formed as a result of the action of the materials from which the implants are made [26], induce in parallel the synthesis of pro-inflammatory cytokines, including IL1-beta, thus leading to the amplification of the inflammatory process in the context peri-implantitis [27,28]; other studies have demonstrated that in high concentrations, stimulated by titanium nanoparticles, free radicals block the synthesis and functionality of osteoblasts, with the consequent degradation of collagen and the activation of the osteolytic process [29,30], dysbiosis and consequent inflammation [31].

Nitric oxide was quantified in saliva, an easily collected, non-invasive biological

product, which is known to have a chemical composition and analyzes similar to those of blood, properties for which saliva is preferred as a biological product for evaluating the state of health/disease of the body human [32,33]. Being an unstable gas, with a short half-life (0.1-10 s), the assessment of NO production was practically, indirectly, by quantifying the oxidized forms of NO, respectively, nitrite NO₂⁻ and nitrate NO₃⁻ [34,35]. That's why the NO bands fold on this principle, indirectly relevant to the NO concentration, by evaluating nitrites based on the principle of the Greiss reaction-colorimetric test, which classically quantifies nitrites in saliva [36]. The evaluation of NO by Berkeley strips is a simple, fast and non-invasive method, validated, there are comparative studies between the values of nitrites by two methods: the Berkeley test strip, CA, USA and chemiluminescence; thus, the studies carried out by Abrar M. Babateen et al. identifies a significant positive correlation regarding the nitrite values quantified by the Berkeley strip ($r=0.830$) and chemiluminescence ($r=0.770$) [37]. Similar studies were also carried out by Ashwin Modi et al., who validated two types of strips for the quantification of salivary nitrites as markers for NO, respectively the strips produced by the companies Berkeley and Neogenesis, comparing the results obtained on these strips with the standard methods used for the quantification nitrites and found that the results correlate positively ($r=0.760$ for the Berkeley strips and $r=0.590$ for the Neogenesis strips) [38]. These observations allow us to appreciate that the performance of salivary strips for the assessment of oxidative

stress in the context of peri-implantitis is a method that can be easily used in dental offices, as a marker for diagnosis and monitoring of this condition. The results of the present study show that there are statistically significant differences regarding the values of salivary nitric oxide between the groups of patients with a favorable evolution after the insertion of dental implants and the patients with mucositis and peri-implantitis both at the first determination and at the second, made after 3 months with the exception of patients with mucositis and peri-implantitis where there are no statistically significant differences regarding the quantified nitric oxide values. These data demonstrate the fact that NO can only be used to delimit the favorable and unfavorable state of evolution after the insertion of dental implants without being able to delimit mucositis from peri-implantitis. Also, there is a correlation between the nitric oxide values and the peri-implant groove in all three study groups (favorable evolution: $p = 0.211$, $r = 0.342$); (mucositis: $p = 0.001$, $r = 0.676$); (peri-implantitis: $p = 0.05$, $r = 0.650$) for both after one month and at the determination after 3 months after the insertion of dental implants (favorable evolution: $p = 0.57$, $r = 0.156$); (mucositis: $p = 0.0004$, $r = 0.725$); (peri-implantitis: $p = 0.004$, $r = 0.844$), which allows us to appreciate that NO can be used to estimate the clinical status of patients after the insertion of dental implants, without being able to appreciate the severity of the peri-implant destructive phenomena. Results similar to those obtained in this study regarding NO values were cited by other authors who identified an increase in NO

levels up to 3 weeks after the insertion of dental implants, as a result of the oxidative stress generated by bone remodeling processes and a return to basal values after 8 weeks [39]. The results obtained in the present study allow us to appreciate that the quantified NO is largely the proof of the oxidative stress generated after the insertion of dental implants. The limits of this study are related to the fact that there are other sources of nitrites, namely nitrate from food, which is metabolized by the bacteria in dental plaque into nitrites and then into NO, given that the patients in the study did not have a restrictive diet regarding the nitrate content of foods before performing the tests. Salivary concentrations of NO are limited by physiological processes, but are strongly affected especially by pathological processes in the oral cavity, in which large amounts of NO are generated. With all these limits, the existence of differences in NO levels related to the three clinical statuses studied, their correlation with clinical parameters and the decrease in NO values in parallel with clinical improvement allows us to appreciate that oxidative stress, in the context of complications after the insertion of dental implants, can be appreciated by quantifying NO levels. Also, the obtained results support the fact that the correct management of the oxidative stress generated after the insertion of dental implants is extremely important, given that it has been demonstrated that oxidative stress indirectly conditions the osseointegration and long-term stability of the implant [40]. All these observations support the expansion of studies on the contribution of N free radicals in the etiopathogenesis of

peri-implantitis and thus to the completion of the list of biomarkers useful in the diagnosis and monitoring of this condition. Also, the test used is fast, reliable and reproducible, qualities that recommend it in the evaluation of the level of oxidative stress in the diagnosis of peri-implantitis, but also of other oro-dental diseases in which oxidative stress is involved. The development of strategies to limit the effects of NO, the elimination of peroxynitrite with all the devastating effects demonstrated otherwise and in other inflammatory processes at a distance from the oral cavity (cardio-vascular diseases, stroke, diabetes, cancer), will be crucial elements in improving the health of mankind [8]. Specialists in the field appreciate that there is currently no clear standardized biomarker for peri-implant diagnosis; in these conditions, the clinical diagnosis supported by biomarkers could provide more precise information regarding peri-implantitis, at the same time compensating some limits of the clinical parameters. Also, we consider that along with the study of biomarkers, the development of new innovative technologies in implantology and the use of biomaterials with superior properties regarding antibacterial and antioxidant effects are objectives that research in implantology must reach as soon as possible [40].

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Conclusions

Within the limits of the studied groups, NO can be considered a marker for estimating the inflammatory process in the context of peri-implantitis. The facility with which NO is determined in saliva, recommends that the test be used in the dental office as a biomarker in the diagnosis and monitoring of peri-implantitis. Salivary NO can be considered a biomarker for evaluating the oxidative processes involved in possible complications after the insertion of dental implants.

Funding

This research received no external funding.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Bio-Ethics Committee of "Ovidius" University (11/10.04.2023).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflict of interest

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