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OXYGEN AND NITROGEN REACTIVE SPECIES IMPLICATIONS IN THE ETIOPATHOGENESIS OF THE PERIODONTAL DISEASE

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Abstract. Reactive oxygen species (ROS) are involved in physiological and pathological processes either as trigger agents or, more frequent, accompanying and/or aggravating the primary lesions. The authors observed the changes of some oxidative stress markers (lipoperoxides, free malondiladehyde) and of some antioxidant markers (ceruloplasmin and hydrogen-donors capacity) in mixed unstimulated saliva of patients with periodontal disease. The results had been compared with those of a witness group consisting in persons with no oral lesions or other diseases that can induce oxidative stress. Through the analysis of the results was observed a significant increase of the lipids peroxidation in parallel with a decrease of the antioxidant defense systems. The authors demonstrated, also, an NO increase in mixed saliva of patients with periodontitis, an observation that pleads for the implication of the nitro-oxidative stress in the inflammatory and degenerative lesions of the periodontal tissue.

INTRODUCTION

Oxygen, an indispensable element for the life, is also the source of some active species with harmful and destructive potential – reactive oxygen species (ROS), species that manifest their effect either when their production increase, or when the "antioxidant" defense systems are overwhelmed, the result being the onset of the oxidative stress (OS) (Dejica, 2000).

Due to its frequency, gravity and, foremost, to its bio-psycho-social implications, the periodontal disease represents an important problem in pathology, that needs a large team of practitioners in order to determine the correct diagnosis, prophylaxis and treatment. In the various etiopathogenesis of the periodontal pathology, besides the inflammatory, immune, microbial, local or general vascular processes and environmental factors (alimentation, smoking and stress) are also involved reactive oxygen (ROS) and nitrogen species (RNS) – either as trigger agents or, more frequently, aggravating the primary lesions (Halliwel, 1991).

Oxygen and nitrogen reactive species are involved in a large number of physiological and pathological processes. ROS generated by monocytes and neutrophyles during inflation are important aggression factors on the paraodontal tissue. ROS play an important role in the activation of osteoclasts and in bone resorption (Wactawaski-Wende, 2001). During oxidative aggression generated by these metabolites the cells defend themselves by producing antioxidant agents, glutathione, cathalase, superoxid-dismutase (SOD) and nitric oxide (Dejica, 2001).

During inflammatory processes large quantities of oxygen peroxide (H_2O_2) are generated and overwhelm the local cellular defense mechanisms; tissue damages appear and increase the chemotaxis and the activation of the leucocytes with consequent liberation of prostaglandins and leukotriens.

Nitro-oxidative stress represents all the oxidative damages induced by ROS and RNS on the cell membranes, with consequences on the entire organism.

The saliva, due to its increased amount of oxidant and antioxidant substances, represents the first defense line, found in the oral cavity, against ROS and RNS, preventing the onset of the nitro-oxidative stress, which may induce periodontal lesions (Preshaw et al., 2004; Popa, 2001).

Our study was focused on underlining ROS and RNS implications in the etiology and pathogenesis of the periodontal disease, with further impact on its evolution, prophylaxis and local and general treatment (Albandar, 2005; Gsfar & Iliescu, 1998).

We intended to observe the changes in the amount of free radicals in mixed saliva on patients with periodontitis, before the onset of a specialized treatment (medical or surgical).

MATERIAL AND METHOD

We studied twenty five patients suffering of chronic periodontitis. Patients repartition according to sex and age intervals is represented in fig. 1. We also had a witness group consisting of fifteen "healthy" subjects (age 18-29), that had no periodontal lesions. The patients with periodontitis had no other disease and didn't follow any treatment that may induce changes in the oxidants-antioxidants equilibrium.

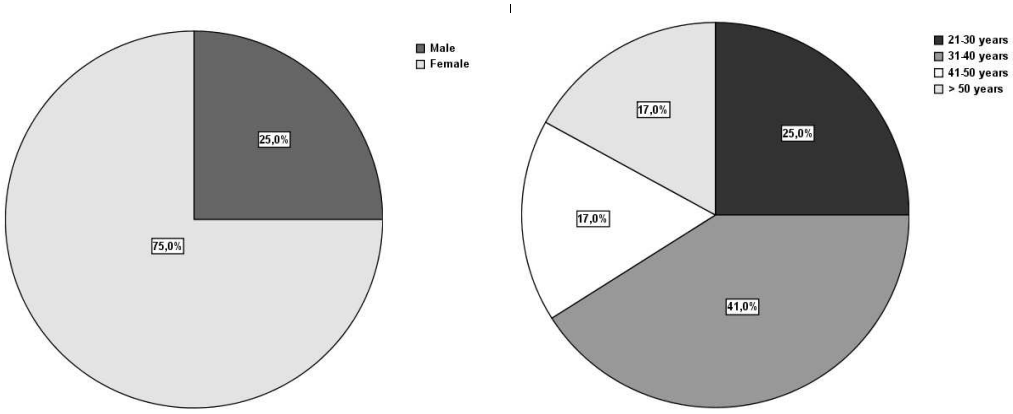

Fig. 1 – Patients repartition according to sex and age intervals

From all the subjects had been taken, in test tubes, samples of unstimulated mixed saliva. The samples were taken before the onset of the medical or surgical treatment. After that, the saliva samples had been freeze at -20^{o}C.

The oxidative stress markers were appreciated in the Oxidative Stress Laboratory of the Physiology Department of "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca. The following oxidative stress markers were assessed: free malondiladehyde (MDA), using Eserbauer method (1994) and lipoperoxides, using Satoh method (1978); the results are expressed in nmol/ml. Also, the following markers of the antioxidant defense had been assessed: the ceruloplasmin, using Ravin method, and hydrogen-donors capacity, using Janasewska method; this method appreciates the concentration of glutathione, cysteine, vitamin C, glucose in biological samples, and the results are expressed in percents. The salivary NO level was determined using Gries method.

RESULTS AND DISCUSSIONS

The saliva plays an important role in the local antioxidant defense due to its enzymatic and non-enzymatic systems, between which we count the ceruloplasmin. Analyzing the

results, we observed a significant decrease of its value in patients with periodontitis (3.85±2.83) in comparison with the witness group (4.88±2.65) (Fig. 2). This decrease of the ceruloplasmin level in the mixed saliva of the patients with periodontal disease may be induced by its "consumption" in the neutralizing reaction of the reactive oxygen species.

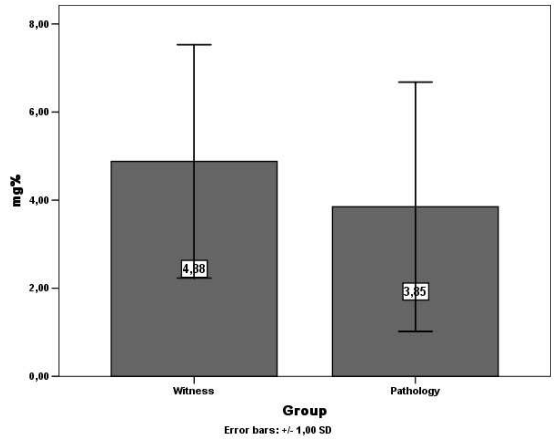

Fig. 2 – Mean values of salivary ceruloplasmin

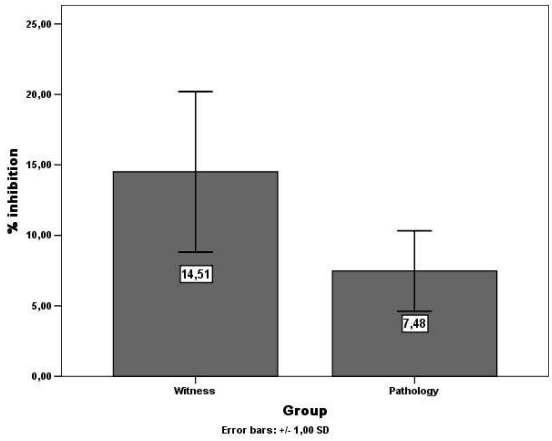

Fig. 3 – Mean values of salivary hydrogen-donor capacity

A statistical significant decrease of the hydrogen-donors capacity was observed in patients with periodontal disease (7.48±2.85%) in comparison with the persons in the witness group (14.51±5.7%) (Fig. 3). The decrease of the hydrogen-donors capacity in the unstimulated mixed saliva can be interpreted as a proof for the existence of the antioxidant defense in patients with periodontal disease.

Our research showed a statistical significant increase of the nitric oxide in the patholgy group (28.85±4.44nmol/ml) in comparison with the person in the witness group (9.19±1.14nmol/ml) (Fig. 4).

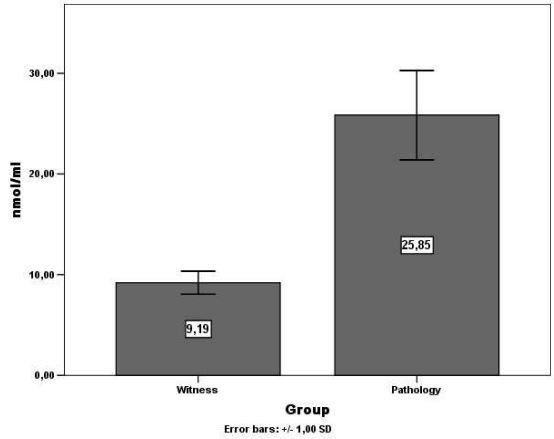

Fig. 4 – Mean values of salivary NO

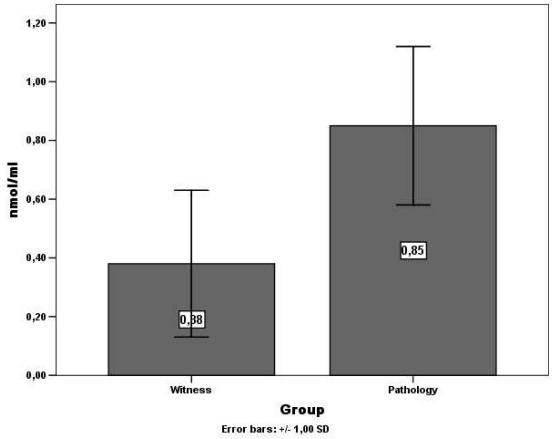

Fig. 5 – Mean values of salivary lipoperoxides

Also, there was a statistical significant increase of the salivary lipoperoxides in patients with periodontal disease (0.85±0.27nmol/ml) in comparison with the witness group (0.38±0.25nmol/ml) (Fig. 5).

At the dosage of salivary MDA we found higher values in patients with periodontal disease (4.42±1.04 nmol/ml) than in the witness group (3.13±0.49 nmol/ml) (Fig. 6). The significant increase of the salivary lipoperoxides, corroborated with the changes in the MDA level are a proof of the oxidative stress implication in the inflammatory and degenerative lesions of the oral cavity.

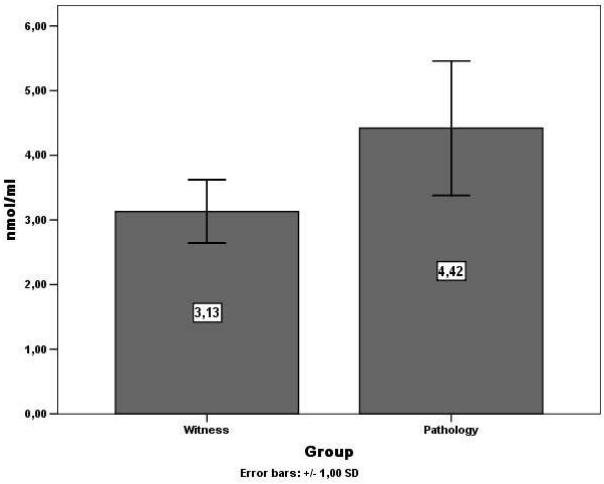

Fig. 6 – Mean values of salivary malondiladehyde

The periodontal disease leads to a precocious increase of the nitro-oxidative stress that demonstrates the presence of an acute inflammation associated with the chronic specific one.

The last years' studies showed that ROS and RNS are involved in the very complex etiopathogenesis of the periodontal disease. ROS interacts with a variety of cellular and extra-cellular protein molecules (Reznick & Parcker, 1994), lead to the peroxidation of lipids, damage cell membranes and liberates intracellular components, destroy the components of the epithelial membrane and lead to changes of the cell metabolism. In their turn, these products modulate the inflammatory reactions, either by inactivating the mediators of the inflammation (C_{5a}, prostaglandins, LTC_4, chemotactic peptides), or by stimulating the release of mediators from platelets and mast cells. Peroxyl radical can react with the membrane lipids in order to form lipoperoxides (Reiter et al., 2000). Under the action of leucocitary peroxidases, H_2O_2 is the substrate that leads to the generation of toxic products. The mieloperoxidase activates cytotoxic and immune-modulating mechanisms.

During acute inflammation, the NO generated by NOS is a mediator of the unspecific defense, the NO, together with ROS, having a cytotoxic and cytostatic effect on the bacterial agents (Olinescu, 1994). In the inflammatory cells it is stimulated the generation of NO and of superoxide anion ($O_2^{\bullet -}$). NO reacts with $O_2^{\bullet -}$ and other ROS, leading to the generation of RNS. Paradoxically, ROS and RNS take part in the regulation of some physiological processes, but, on the same time, they destroy non-self and self cells. The impact of their chronic synthesis on the paradontium has consequences unrelated with their synthesis rate and with the composition of the environment in which this process takes place (Jeney & Pârvu, 2006).

In periodontal disease was observed an increase in the NO synthesis rate in the remaining cells and, also, in the leucocytes. The NO excessive local generation has, on one hand, a benefic effect, leading to the destruction of the bacteria, but, on the other hand, a harmful effect, by stimulating the inflammation and the bone resorption. That's why, on short term, the NO synthesis inhibition may have benefic effects (Brock et al., 2005).

CONCLUSIONS

1. Malondialdehyde and lipoperoxides have significant increased values in patients with periodontal disease, suggesting the involvement of the oxidative stress.

2. We observed a significant decrease in the concentration of salivary ceruloplasmin and in the hydrogen-donors capacity, which shows a decrease of the antioxidant defense systems from saliva.

3. We found a statistic significant increase of the nitric oxide in mixed saliva of patients with periodontal disease, which is the expression of the involvement of the nitro-oxidative stress.

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