Correlation between histopathological aspects of periodontitis and biochemical changes of oxidative stress

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Abstract

Purpose: The aim of this paper is to assess the histopathological changes and the condition of oxidative stress in the saliva of the patients with periodontal disease.

Materials and Methods: We have carried out our research on two groups of patients with periodontitis (a group of 16 patients with stage I periodontitis and a group of 16 patients with stage II periodontitis), who we have compared, using samples of saliva and serum, to a group of 15 volunteers without any dental restorations. To dose MDA, we used thiobarbituric acid method and for determination of ceruloplasmin the Ravin’s method. For histopathological examination, the periodontal fragments were immediately underwent for fixation in 10% neutral formalin solution and then, they were included in paraffin using the conventional histological technique.

Results: The group of patients with stage II periodontitis presented lower values of salivary MDA compared to the group of patients with stage I periodontitis. Patients with stage I periodontitis presented a significantly increased concentration of MDA in the serum compared to the control group, but considerably lower concentrations compared to patients with stage II periodontitis. The values of serum ceruloplasmin do not present statistically important changes in patients with stage I periodontitis compared to those suffering from stage II periodontitis and to those from the group control.

Conclusions: MDA is not a product of blood filtration as the concentration of MDA in the bloodstream is bigger than in the saliva. Tissue changes and biochemical aspects are strongly connected. Removing dental plaque can prevent oral infections.

Keywords: periodontitis, reactive oxygen species, ceruloplasmin.

\section*{Introduction}

Several reactive oxygen species (ROS) and lipid peroxidation products are produced in physiological amounts in the human body, but it is well known that overproduction of ROS occurs especially in chronic inflammation [1]. The human body has many antioxidant defense mechanisms (enzymatic and non-enzymatic antioxidants) which removes ROS when it is produced and prevents their harmful effects [2].

During the last few years, a great attention has been given to the role of reactive oxygen species, to lipid peroxidation products and antioxidant systems in the pathology of periodontal disease.

Chronic periodontitis, also known as adult periodontitis, is an infectious inflammatory disease caused by the bacteria from dental plaque, which determines progressive destruction of the tissues that support the teeth (e.g., the gum, the periodontal ligament, cementum, and the alveolar bone). Periodontal disease consists of periods of exacerbation interspersed with periods of remission and presents a local microbial burden that initiates local inflammation and local tissue destruction [3].

During experimental periodontitis, it has been noticed an increased lipid peroxidation and a decreased antioxidant activity. Chapple IL et al. have demonstrated that total antioxidant activity has suffered a greater reduction in the saliva of patients with periodontal disease than to those without periodontitis [4].

Our research was aimed to assess the histopathological changes and the condition of oxidative stress in the saliva of the patients who suffer from periodontal disease.

\section*{Materials and Methods}

We have studied:

- 16 patients suffering from stage I periodontal disease (hospitalized at Department of Orthopedics, Oradea County Hospital, Romania) aged between 30–50 years;
- 16 patients suffering from stage II periodontal disease (hospitalized at Department of Surgery, Oradea County Hospital) aged between 30–70 years.

The assessment of oxidative stress has been conducted on samples of serum and saliva. The oxidative aggression has been appreciated by dosing MDA with thiobarbituric acid and ceruloplasmin using the Ravin’s method. The results have been compared to a control group of 15 volunteers without any dental restorations.

\section*{Microscopic examination}

For histopathological examination, the biological material collected, represented by periodontal fragments,
were immediately underwent for fixation in 10% neutral formalin solution. Periodontal tissues were fixed for 6–12 hours. Then, they were included in paraffin using the conventional histological technique. Four µm thick sections were cut using a microtome. Sections were stained with Hematoxylin–Eosin (HE), Masson’s trichrome, Van Gieson and Reticulin stain. All slides were viewed under a Nikon E600 microscope and the photographs were taken with a Nikon camera.

**Statistical analysis**

Data were expressed as mean ± SD (standard deviation). The significance of the results was assessed by the Student’s t-test. A p-value <0.05 was considered statistically significant.

**Results**

The research conducted on patients with stage I periodontitis has shown a significant increase of salivary MDA (from 0.58±0.14 nmol/mL for control group, to 2.17±0.55 nmol/mL for patients with stage I periodontitis) (p<0.001). Also, the group of patients with stage II periodontitis presented a statistically important increase of MDA (from 0.58±0.14 nmol/mL for control group, to 2.05±0.48 nmol/mL for patients with stage II periodontitis) (p<0.001). The group of patients with stage II periodontitis presented slightly lower values of salivary MDA compared to the group of patients suffering from stage I periodontitis (from 2.17±0.55 nmol/mL to 2.05±0.48 nmol/mL) (p>0.1) (Figure 1).

Salivary ceruloplasmin to the group suffering from stage II periodontitis has much lower values in comparison to the control group (from 3.46±1.25 mg% for the control group, to 1.11±0.66 mg%) (p<0.001). Regarding patients with stage I periodontitis, the ceruloplasmin concentration is low but not significantly (from 3.46±1.25 mg% for the control group, to 2.14±1.18 mg%) (p>0.1). Compared to the group suffering from stage II periodontitis, those with stage I periodontitis have presented much lower values of ceruloplasmin, but statistically non-significant (p>0.1) (Figure 3).

Patients suffering from stage I periodontitis presented a significantly increased concentration of MDA in the serum compared to the control group, but considerably lower concentrations compared to patients with stage II periodontitis (from 1.91±0.33 nmol/mL for group control, to 2.62±1.31 nmol/mL for patients with stage I periodontitis) (p<0.001) (from 4.12±0.41 nmol/mL for patients suffering from stage II periodontitis to 2.62±1.31 nmol/mL for the patients with stage I periodontitis) (p>0.1). The group of patients with stage II periodontitis has shown significantly increased values of MDA compared to the control group (from 1.91±0.33 nmol/mL to 4.12±0.41 nmol/mL) (p<0.001) (Figure 2).

The values of serum ceruloplasmin do not present statistically important changes in patients with stage I periodontitis compared to those suffering from stage II periodontitis and to those from the group control (p>0.1) (Figure 4).
**Histopathological results**

In Figure 5, we can observe an acute inflammatory response characterized by dilatation of small blood vessels, enhanced polymorphonuclear migration and increased vascular permeability, which manifests as an increase in the flow of crevicular fluid.

The earliest histological evidence of the progression of gingivitis to periodontitis is the extension of inflammation beneath the base of the junctional epithelium into the supra-alveolar connective tissue (Figure 6). The inflammatory infiltrate is rich in plasma cells but there are also numerous T-lymphocytes and macrophages.

Areas of irregular hyperplasia and rete ridge formation (Figure 7) alternate with areas of thinning and even frank ulceration. The vessels in the subjacent connective tissue are markedly dilated (Figure 8) and a large number of PMN are migrating.

The underlying proliferative connective tissue (Figure 9) is densely infiltrated by inflammatory cells (mainly plasma cells) and granulation tissue with abundant new vessels (Figure 10).

The overgrowth of the gingival layer consists mainly of bundles of collagen fibers but fibroblasts are, also, common (Figure 11) and there is a scattered chronic inflammatory cell infiltration. The surface of the epithelium is also often markedly hyperplastic (Figure 12) and it shows long slender rete processes extending into the underlying connective tissue and multiple collagen fibers (Figure 13).
Discussion

Periodontal disease is an inflammatory reaction caused by subgingival bacteria, which determine irreversible tissue damages and tooth loss. Periodontitis affects 7% to 15% of adult population and it is caused by multiple factors, which must include the microbial factor but only the bacteria cannot determine the disease. The connection between environmental factors, behavior, genetic factors and, also, the immune and inflammatory response of the host together with the variations of inflammatory mediators is not fully elucidated [5, 6].

The composition of saliva reflects the nature and the amplitude of the host response to the periodontal microbial challenge. Therefore, determining the levels of salivary components represents a reasonable presumed approach in order to establish the diagnosis and the disease evolution [7, 8].

Reactive oxygen species are involved in periodontal destruction during inflammatory periodontal diseases. The
imbalance between the oxidant and antioxidant activity can represent a key factor in the damaging effects of ROS [9].

The saliva has a great antioxidant capacity and oxidative stress can appear because of lipid peroxidation and of a decreased antioxidant capacity of the saliva [10, 11]. The oxidation of lipids can be easily done throughout the organism because the cell membrane consists of two layers of lipids containing from place to place proteins, which perform the role of receptors and channels. A stable MDA is one of the components that results from the reaction and it is considered a loyal pointer of the level of oxidation throughout the body.

During stage I and II periodontitis, salivary MDA suffers an important increase but far more marked in the bloodstream, argued by the fact that MDA is not a plain blood filtration product in saliva.

Considering the results, it can be observed an increased oxidant activity in saliva and blood reflected by the increased concentration of MDA and by the decreased concentration of ceruloplasmin. Ceruloplasmin reduces the amount of ROS, acting like a non-enzymatic antioxidant. It is a α2-globuline, exclusively produced in the liver and eliminated via the biliary route [12].

Among patients who suffer from early, moderate or advanced periodontitis, only those with advanced periodontitis experienced a reduced total antioxidant activity and high levels of reactive substances with thiobarbituric acid, as a marker of the presence of reactive oxygen species. The existence of oxidative stress in advanced periodontitis and its absence in early and moderate periodontitis can explain the discrepancy between previous studies [13].

Examining the histopathological sections, we can observe typical inflammatory lesions for each stage of marginal periodontitis and adaptive changes (except for those destructive) of the gingival epithelium and chorion.

At the debut of this disease (the stage of gingivitis), marks of acute inflammation can be revealed on the histological sections: congested and dilated vessels, leukocyte migration and inflammatory elements (especially PMN) which invade the chorion.

If the periodontal disease extends, we can notice chronic inflammatory characteristics such as granulation tissue, new-formed vessels, an increased number of conjunctive fibers in chorion, inflammatory infiltrate composed by lymphocytes and plasma cells associated with macrophages and histiocytes. Because of both mechanical (dental plaque) and biochemical (oxidative stress) aggressions, the superficial (keratinisation) and spinous layer (acanthosis) of the stratified squamous free-keratin epithelium of the periodontium suffer several changes.

During the last histological stage of marginal periodontitis (chronic gingival hyperplasia), fiber changes in the chorion, papillomatous hyperplasia of the gingival epithelium along with acanthosis, koiocytosis and superficial keratinisation can be observed.

The neutrophils can contribute to tissue destruction by producing ROS, enzymes and cytokines, which augment the inflammatory response [14, 15]. The main source of ROS in the periodontal disease is the respiratory explosion caused by the PMN activation.

The activated PMN is a great source of IL-1 and TNF-α and these cytokines play an important role in inflammation and during the repairing processes.

The presence of congested and dilated vessels, the vascular endothelium with a high permeability, the migration of leukocytes, the chorion invaded by inflammatory elements (especially PMN) during the initial stage of the periodontal disease and the increased number of conjunctive fibers in the chorion as the disease evolves, the inflammatory infiltrate composed by lymphocytes and plasma cells are microscopic aspects of inflammation which sustain the increased salivary concentration of MDA to these patients.

We recommend continuing the research in order to find an adequate antioxidant therapy for periodontal disease.

Conclusions

During stage I and II periodontal disease, the concentration of MDA in saliva is significantly increased, but more important in the bloodstream, arguing that MDA is not a product of blood filtration. The pathological process in the marginal periodontal tissue starts with an acute inflammatory response with tendency to a chronic inflammation and degenerative lesions. There is a connection between tissue changes and biochemical aspects. It can be stated that it is the responsibility of dental professionals to ensure that all oral infections are kept to a minimum in order to stop the formation of dental plaque.

References


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