CORRELATIONS BETWEEN THE PERIODONTAL MODIFICATIONS AND LIPID PEROXIDATION IN PERIODONTAL DISEASE PATIENTS

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ABSTRACT

The purpose of the study Our study was centred on the comparative research of enzymatic and non-enzymatic oxidative status mediators in the crevicular fluid on chronic and aggressive periodontitis patients, compared to periodontal healthy subjects. **Materials and methods** The study was conducted on 42 patients. They were divided in 3 study groups: chronic periodontitis subjects, aggressive periodontitis patients and periodontal healthy subjects. On marginal periodontitis patients, associated or not with systemic diseases, we initially collected gingival crevicular fluid and saliva samples and we evaluated the following enzymatic stress markers for all the three groups of subjects: superoxide dismutase, glutathione peroxidase, malondialdehyde, using the gingival crevicular fluid (GCF) as biologic material. **Results** The statistical analysis for the malondialdehyde in the control group, aggressive periodontitis group and chronic periodontitis group shows significant differences between the groups. SOD values in the gingival crevicular fluid were low in the periodontitis groups compared to the control group. Despite the strong correlation between the glutathione peroxidase values in the three groups, we did not observe any significant differences between the GPx values in the aggressive periodontitis, chronic periodontitis and control groups. **Conclusions** The present study revealed significant statistic differences between most of the oxidative stress parameters analysed in the GCF, especially in the aggressive periodontitis group, less in the chronic periodontitis group compared to the control group.

Key-words: oxidative status, chronic periodontitis, aggressive periodontitis

INTRODUCTION

A thorough and recent analysis concluded that the oxidative stress is the main determinant in the periodontal tissue destruction resulted from the host-microorganisms interactions [1]. Tissue damage results from the processes determined by the chronic inflammation, as a consequence of the phagocyte mobilization and activation [2, 3].

The pathogenic relationship between the chronic inflammation and the oxidative stress is still a puzzle not yet completely understood. The inflammation could induce the oxidative stress which in turn could enhance the inflammation by activating a series of nuclear transcription factors such as NF-κB, that are modulators of certain gene expression responsible of some pro-inflammatory mediators generation.

Both factors could be contributing to the chronic disease [1, 4].

The excessive production of pro-inflammatory cytokines, proteinases and

reactive species (oxygen or nitrogen derived reactive species) enhance the chronic inflammatory lesions [5].

The oxidative stress can determine the periodontal tissue lesions in a direct manner, by oxidation of important biomolecules or, indirectly, through activating the transcription factors that are involved in redox reactions (such as nuclear factor - NF-κB) that lead to a distant reaction of pro-inflammatory factors genes, possible associated with a systemic response [4, 6, 7].

Most of the research studies in the field suggest that an optimal balance between the free radicals of oxygen reactive species type and the antioxidant levels is essential in many normal biological processes, while low doses of certain radicals or radical derived species can stimulate the fibroblastic proliferation in vitro [8, 9]. A low level of free radicals can also act as an inductive signal, the high levels being involved in tissue destruction [10, 11].

The interactions between the disease course, other potential factors (like cigarette smoke, systemic disorders) and the genetic control of the modifying factors are included in the determination of specific clinical manifestations of the disease [11, 12, 13].

Objective

Our study aimed to assess the oxidative stress profile in patients with two different periodontal alterations, using gingival crevicular fluid samples, and to compare this biomarker in a group of healthy subjects. The oxidative status was evaluated through comparative analysis of malondialdehyde – MDA, a measure of lipid peroxidation level within gingival crevicular fluid of chronic and aggressive periodontitis patients, compared to periodontal healthy subjects.

MATERIALS AND METHODS

A total number of 42 patients (22 male and 20 female), aged between 24-55 years, selected from the patients of the Periodontology Clinic – University of Medicine and Pharmacy "Gr. T. Popa" Iaşi and examined between May 2012 and November 2012 were enrolled in our study.

The inclusion criteria consisted in subjective manifestations (gingival pruritus, physiognomic impairments, and masticatory deteriorations) and especially objective clinical expressions (gingival bleeding, gingival recessions, dental mobility, and periodontal pockets).

We also selected patients with associated systemic disorders, n=11 with diabetes mellitus and n=8 experiencing dyslipidaemia.

The enrolled patients were included into 3 different study batches:

- Group A with chronic periodontitis (CP) – (n=16)
- Group B with aggressive periodontitis (AP) – (n=11)
- Group C with periodontal healthy subjects – (n=15)

For every subject included in the present study, informed and written consent form was taken, and clinical examination was performed in the dental office. The evaluation consisted in registrations of various periodontal indexes: periodontal probing with WHO periodontal probe, bleeding index, oral hygiene index (plaque index-PI). The patients were informed regarding the necessity of periodical evaluation and the importance of periodontal impairment in the course of potential systemic diseases.

The exclusion criteria were as follows: immune-deficiency patients, pregnant and lactating female subjects, patients during orthodontic or odontal-periodontal treatments or the ones subjected to such treatments in the last 12 months, patients under antibiotic therapy within the last 6 months.

Gingival crevicular fluid was collected by a standardized method and the level of lipid peroxidation (amount of malondialdehyde) was assessed by spectrophotometric procedure.

Other routine biochemical parameters (useful for assessments of the systemic condition) were evaluated after careful venous blood collection. The serum obtained after centrifugation, was subjected to an automatic analysis with COBAS INTEGRA 400-700-800 device.

We analysed the blood sugar level, glycosylated haemoglobin and lipid profile in the peripheral blood.

The gingival crevicular fluid collection technique

The GCF was collected in the dental office by the least aggressive technique, avoiding any mechanic irritations.

The sample prelevations took place after the evaluation of plaque index, bleeding index, calculus index and a thorough control of bacterial plaque (professional brushing), isolation from saliva and air drying for the gingival sulcus. Special paper strips (Periopaper ProFlow Inc, Amityvile, NY) were introduced within the gingival sulcus for 30 seconds [4, 5]. The blood contaminated strips were discarded. After the sample collection, a quantitative analysis was conducted using a very fine Periotron 8000 device.

The samples were loaded in phosphate buffer (PBS) 250µl, pH 7.4 on 40C and vortexed. From the gingival crevicular fluid solved in PBS samples of 100µl were provided for photometric determinations.

Protocol for the lipid peroxidation mediator analysis

Considering the potential of etiopathogenic agents for triggering the impairment of the lipid equilibrium of the membrane, we've selected from the high range of GCF mediators exclusively malondialdehyde, which should express the lipid peroxidation level, as part of oxidative profile.

According to most of the recent studies, the free radical formation can extensive impair not only proteins and genetic material, but also lipids. Previous studies pointed out that MDA in body fluids can act as a biomarker of oxidative stress in certain disorders, including also chronic inflammatory diseases. Moreover, as a result of reactive species interaction with fragile unsaturated fatty acids within cell membranes, a deleterious process of lipid peroxidation can be initiated and developed, MDA (a low molecular weight end product) being thus an indicator of lipid peroxidation, with various harmful impact on biological systems.

Malondialdehyde was determined by spectrophotometric measurements in the gingival fluid by the method described by Jain et al, according to the manufacturer protocol [15].

The obtained data were introduced in the statistical analysis program (SPSS 12) and estimated by the statistical functions (t-student test). In each case, p value < 0.05 was considered statistical significant.

RESULTS AND DISCUSSIONS

Clinical evaluation of the patients pointed out the following aspects: 26% of the subjects presented aggressive periodontitis, 38% of the subjects being affected by chronic periodontitis, while 36% of the subjects were periodontal healthy, and constituted the control group.

In the chronic and aggressive periodontitis groups, 16 subjects were male and 11 female. The smoking percentage presented as follows: 59% smoking males and 57% smoking females.

Regarding the oral hygiene status, the statistical data revealed a PI mean value of 34.96±6.72, with a maximum value of 65.20 %. A mean value of 34.21±5.78 was recorded for the calculus index, with a maximum value of 60.57% (Table 1). Both maximal values were found among persons belonging to the rural area.

The statistical analysis for the periodontal indexes revealed a strong statistical correlation between these indexes.

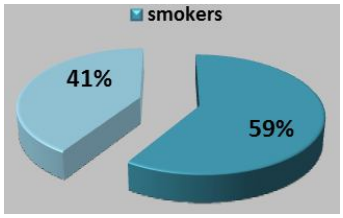

Fig. 1. Proportion of men smokers in the studied groups

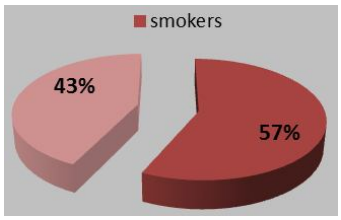

Fig. 2. Proportion of women smokers in the studied groups

Parameter	Minimal value	Maximum value	Standard deviation	Variation coefficient-%
Age	24	55	10,72	114,94.
Plaque Index (%)	8.9	65.2	21.27	452.72
Calculus Index (%)	7.9	60.57	18.28	334.28
Bleeding Index (%)	30.12	85.6	18.29	334.87
Gingival Index	1.23	2.85	0.51	0.27

Table 1. Clinical parameters in the study group

Furthermore, due to the gingival recessions, the physiognomic impairments were found to be more prevalent in patients of 30-49 years old. Almost a third part of the patients included in our study (27.78%) claimed some pain of medium intensity in the morning, associated with a slight sensation of dental egression.

The personal medical history could be suggestive for the identification of possible risk factors for marginal chronic periodontitis compared to marginal aggressive periodontitis.

We examined in each group of individuals, the age for the onset of attachment loss for about 10-50% situses. The frequency of attachment loss distribution on different surfaces was evaluated in time.

Considering the low incidence of aggressive periodontitis in the general population, there is a need for sensitive screening approach, for a diagnostic procedure able of correct periodontal impairment, in order to ensure the achievement of a successful costs/efficiency ratio.

The purpose of the screening would be detection of possible affected subjects who would need further investigations.

The most sensitive method of screening in periodontology is represented by the periodontal probing, that specifically assess the attachment loss.

CAL	*CP*	*AP*
≥ 1 mm	58%	62%
≥ 2 mm	38%	41,5%
≥ 3 mm	22,7%	23,4%
≥ 4 mm	12,3%	11,5%
≥ 5 mm	3,7%	4,8%

Table 2. Comparative prevalence of the gingival recession between the two periodontal impaired groups (CP and AP)

The evaluation of the prevalence and periodontal attachment loss degree (gingival recession, periodontal probing depth) on chronic periodontitis patients in our study, reinforce a direct relationship between the disease and the prevalence of dental calculus.

Oxidative status in the case of periodontal tissue injure was evaluated in our subjects, by means of lipid peroxidation determination, analysing the levels of the lipoperoxidation end product, malondialdehyde (MDA). Being suitable for determination in various body fluids, this short molecule could be easily measured in gingival fluid, offering important data, concerning specific sites of disease, and their degree of oxidative alteration. Thus, the differences in lipid alter mediated through free radicals between the two periodontal affected groups, and each compared to control, could be assessed through MDA evaluation.

	N	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Control group	15	0.36	0.87	.6098	0.04454	0.17252	0.030
Chronic periodontitis	16	0.78	1.43	1.1431	0.05528	0.22111	0.049
Aggressive periodontitis	11	0.82	1.48	1.1364	0.06435	0.21341	0.046
Valid N (listwise)	11						

Table 3. Statistical analysis of MDA in the 3 study groups

Type of periodontal injury	Level of CAL	Variation of MDA levels	
AP		Min	Max
	>1mm	0.82	0.98
	>2mm	0.86	1.07
	>3mm	0.94	1.12
	>4mm	0.98	1.48
	>5mm	1.32	1.48
CP		Min	Max
	>1mm	0.78	0.90
	>2mm	0.85	0.97
	>3mm	0.86	1.28
	>4mm	0.91	1.41
	>5mm	1.32	1.43

Table 4. Comparative analysis between CAL and MDA in the 3 study groups

Statistical analysis revealed increased levels of MDA among periodontal injured subjects, the differences between the study groups being more relevant in the AP group than in the CP group, when compared to control.

The separate analysis of malondialdehyde values in the CP group in the SPSS test pointed out a statistical significance of the values in chronic periodontitis group and aggressive periodontitis group when compared to the control group ($p<0.0001$); no statistical significance in the level of lipoperoxidation between the two periodontitis groups (CP and AP) could be noticed.

The correlation analysis between the bleeding index and malondialdehyde level in aggressive and chronic periodontitis groups revealed a strong correlation between this index and the MDA crevicular level, consistent with the degree of the lipidic peroxidation in these groups.

Moreover, there was a clear association between level of attachment loss and gingival fluid value of lipoperoxidation end product, the highest MDA values occurring in patients that recorded attachment loss> 4mm, 5mm, within both, patients with AP and CP.

The clinical diagnosis is based mainly upon the information achieved by clinical examination of the periodontal tissues; the purpose of the diagnostic is to identify the aggressive and chronic periodontitis patients and to ascertain potential factors that could impact the treatment plan or course of the diseases.

This information offers partially data required for a clear diagnostic of aggressive and chronic periodontitis; they further charge clinical and interrogatory data through advanced methods that could allow accurate diagnostic, therapy and evaluation of the disease [2, 14].

Several studies suggest that a right balance of the free radicals and oxygen reactive species is essential in many normal biological processes and that low doses of certain radicals or radical derived species can stimulate the fibroblastic proliferation an epithelial cells in vitro. A low level of oxygen reactive species can also act as an inductive signal, while higher levels could be destructive [12].

The pathogenic relationship between the chronic inflammation and the oxidative stress is not completely understood. The inflammation could determine the oxidative stress and the oxidative stress could enhance the inflammation by activating a series of transcription factors such as NF-κB and therefore the gene expression for the pro-inflammatory mediators. Both factors could be contributing to the chronic disease.

CONCLUSIONS

The purpose of study was centered on the comparative research of non-enzymatic oxidative status mediators in the crevicular fluid on chronic and aggressive periodontitis patients, compared to periodontal healthy subjects. Despite that we could not identify any statistical difference in the level of MDA between the two periodontitis groups, the separate analysis of malondialdehyde values in the CP and AP group in the SPSS test pointed out statistical significance of the lipoperoxidation level, consistent with the degree of periodontal alteration, when comparing with control, in each of the CP and AP group ($p<0.0001$).

Furthermore, higher local (gingival fluid) MDA levels seem to reflect increased free radicals activity during periodontal inflammation.

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