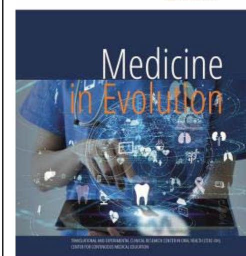
Screening Periodontitis Associated with Diabetes by Using Oxidative Stress Biomarkers in Blood and Gingival Tissue



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Abstract

Introduction: Periodontitis and diabetes have a two-way relationship but the mechanisms implicated in their association are still unclear. Aim and scope: We aimed to evaluate local and circulatory oxidative stress in an experimentally induced periodontitis associated with diabetes on Wistar rats. Materials and methods: Forty Wistar rats were divided in: control group (C) and periodontitis (P), diabetes (D) and periodontitis associated with diabetes (DP) group. Blood and gingival malondialdehyde (MDA) and blood glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ratio, and catalase (CAT), and gingival CAT were determined. Results: blood and gingival MDA increased significantly in P and DP and blood GSH, GSSG, GSH/GSSG, CAT and gingival CAT decreased significantly. Circulatory MDA, GSH, GSSG and GSH/GSSG were significantly modified in DP compared to P. Conclusion: oxidative stress is implicated in periodontitis and periodontitis associated with diabetes and circulatory MDA, GSH, GSSG and GSH/GSSG could indicate a risk of their association.

Keywords: Periodontitis, diabetes, oxidative stress, screening

INTRODUCTION

Although oxygen is one of the most important constituent of the aerobic life, under specific conditions it generates reactive oxygen species (ROS) which are capable of producing necrosis and cellular death. Organisms counteract the accumulation of ROS by augmenting the antioxidant activity. When an imbalance between the prooxidant and antioxidant status occurs, oxidative stress is installed and results into alterations and eventually disease [1,2].

Periodontitis has a multifactorial etiopathogenesis implicating infectious agents, factors depending on the host, different diet habits, genetic factors, the state of the general health as well as the quality of the immune-inflammatory response [3]. The most important etiological factor, the bacterial pathogens in the dental plaque, generate a prolonged inflammatory reaction and systemic conditions that lead to oxidative stress [4-6].

On the other hand, the overproduction of ROS can be strictly associated with hyperglycemia, involving mechanisms like glucose autoxidation, polyol pathway, prostanoids synthesis and protein glycation. Moreover, the exposure of the endothelial cells to increased levels of glucose can cause hyperproduction of ROS [7]. This could explain the periodontal tissues alterations in patients with diabetes in the absence of the dental plaque, and sustain the theory of the interrelation diabetes and periodontitis [8,9].

Even though there is an interrelation between diabetes and periodontitis that goes both directions [9] with diabetes being recognized as a risk factor for periodontitis and periodontitis a risk factor for glycemic decompensation and diabetes [10,11], in present, the mechanisms implicated in the association of periodontitis with diabetes are incompletely elucidated [12].

Aim and objectives

In our research we aimed to evaluated the oxidative stress implication in the periodontitis associated with diabetes pathogenesis, by evaluating the circulatory and local levels of some oxidative stress biomarkers: blood malondialdehyde (MDA), glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ratio, and catalase (CAT) and gingival MDA and CAT in an in vivo model of induced periodontitis in diabetic Wistar rats.

MATERIAL AND METHODS

Experimental protocol

The study was performed at BIOCOM Research Centre of the Department of Physiology, Cluj Napoca, Romania. 40 Wistar albino rats (males, 8 weeks old, having a mean ± SD 220 ± 20 g each) were distributed 5 per cage, at a temperature of 21 ± 2 °C, 70% ± 4% humidity and 12-h dark/12-h light cycle, fed standard pellet laboratory diet and water ad libitum. One week of acclimatization preceded the experiment.

The rats were randomly divided in four groups: Group DIABETES (D) – rats with induced diabetes; Group DIABETES-PERIODONTITIS (DP) - rats with induced diabetes and periodontitis; Group PERIODONTITIS (P) - rats with induced periodontitis; Group CONTROL (C) – control group.

Reagents

We used streptozotocin (STZ), glucose, 2-thiobarbituric acid, o-phthalaldehyde, hydrogen peroxide and kalium phosphate buffer (Sigma Aldrich Chemicals GmbH (Munich, Germany)). The vehicle for streptozotocin and glucose dissolution was distilled water.

Diabetes induction

Under an anesthesia with a cocktail of ketamine (100 mg/kg body weight (bw)) and xylazine (10-mg/kg-1 bw) administered i.m. (intramuscular), diabetes was induced in groups D and DP by a i.v. (intravenous) injection in the caudal vein containing STZ 30 mgkg-1 bw, followed by glucose 30%, 2 mL/animal, at an interval of 6 h [13]. STZ has been found to selectively alter pancreatic β cells by an oxidative stress mechanism, reduce the insulin level and enhance the glucose level in the circulation, leading to diabetes installation [14]. The animals in group P and CONTROL group were given distilled water alone. The rats were tested for diabetes after 72 h, by measuring their glycemia with a glucometer in the blood collected from the caudal vein. A glycemia <300 mg/dL needed the re-administration of STZ and glucose.

Periodontitis induction

For the induction of periodontitis an orthodontic ligature was placed interproximal, in the gingival sulcus around the second molars [13]. The ligature method is the most common method of periodontitis experimental induction [15] as it facilitates biofilm adhesion, plaque formation and bone resorption [16,17]. The depth of the pockets was measured with a dental probe in 6 sites — mesial, central and distal, both on the buccal and the oral side of the tooth. After 15 days, when the deepest pocket reached an average depth of 3.1 mm, periodontitis was declared induced. The ligatures were then removed with the plaque or calculus maintained in place [13].

Blood and Tissue Sample Collection

After 10 weeks, under anesthesia (100 mg/kg bw ketamine and 10 mg/kg bw xylazine administered intraperitoneal in a cocktail), 5 mL of blood was collected from the retro-orbital venous sinus of all rats and biochemical assays were performed. Gingival mucosa of the ligated teeth and control teeth were harvested, homogenized with a polytron homogenizer (Brinkman Kinematica, Lucerne, Switzerland) for 3 min on ice, in phosphate-buffered saline (PBS) (pH 7.4) and added at a ratio of 1:4 (w/v). The suspension was centrifuged at 3000×g and 4 °C for 5 min and from the resulted cytosolic fraction from which the protein content was assessed [18].

Oxidative Stress Biochemical assays

The levels of oxidative stress and antioxidant capacity in blood were measured fluorometrically using 2-thiobarbituric acid in the case of MDA [19] and o-phthalaldehyde in the case of GSH and GSSG [20,21]. The results were expressed in nmoles/mL.

The protein content in the gingival tissue samples was determined with bovine serum albumin as the standard. After the homogenates were heated in a boiling water bath for 1 h in 75-mM K2HPO4, at a pH of 3 and containing 10-mM 2-thiobarbituric acid, they were cooled and the solution was extracted using 3 mL of n-butanol in 0.6 mL of 2-thiobarbituric acid. MDA levels were estimated spectrofluorometrically in the organic phase using a synchronous technique with 534 nm of excitation and 548 nm of emission. MDA was expressed as nmol/mg protein.

For CAT evaluation, the homogenates and cell lysates were put into reaction with 10-mM hydrogen peroxide in 50-mM kalium phosphate buffer, pH 7.4. CAT was quantified in units/mg protein, where one unit was defined as the quantity of enzyme that generated an absorbance reduction of 0.43 at 25 °C per minute at 240 nm [22].

Statistical Analysis

ANOVA with the Scheffe's tests were applied to compare the levels of significance between groups. SPSS 24 software, Armonk, NY, USA was used for all statistical analyses. The values of $p \leq 0.05$ were considered significant.

RESULTS

Blood MDA, GSH, GSSG, GSH/GSSG and CAT in diabetes (D), periodontitis (P) and diabetes associated with periodontitis (DP) were significantly modified when compared with the control group. MDA, the marker of the oxidative stress was enhanced in the induced pathologies and GSH, GSSG, GSH/GSSG and CAT, indicators of the antioxidative activity were reduced (Figure 1).

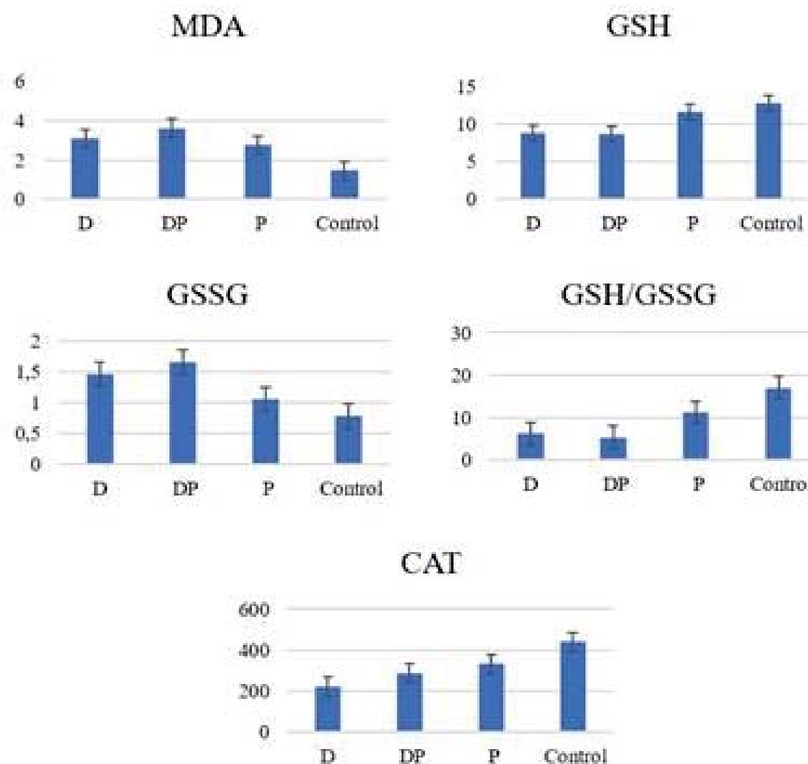

Figure 1. Mean variable evolution of oxidative stress (MDA) and antioxidant capacity biomarkers (GSH, GSSG, GSH/GSSG and CAT) in blood

At the ANOVA test, significant differences of MDA, GSH, GSSG, GSH/GSSG and CAT blood values were depicted between all three groups compared to the control group (Table 1).

Table 1. Variance analysis of the blood variables for groups D, P, DP and Control

	SS	F	p-value
MDA	30.08	33.47	0
GSH	162.464	21.76	0
GSSG	5.36	35.26	0
GSH/GSSG	941.08	52.25	0
CAT	345559	24.63	0

SS—Total Sum of Squares, MDA—malondialdehyde, GSH—glutathione, GSSG—oxidized glutathione and CAT—catalase

The Scheffe test resulted in significant differences between the control group and all other groups, except for the blood GSH levels in DP group.

There were no significant differences in none of the markers when groups D and DP were compared.

Blood MDA was comparable in D and P groups, but the antioxidant capacity biomarkers were significantly different. GSH, GSH/GSSG and CAT were more reduced in diabetes than periodontitis, while GSSG was lower in periodontitis. In the case of diabetes associated with periodontitis (DP), MDA had higher levels than in periodontitis (P) and the antioxidant activity was lowered (GSH, GSH/GSSG and CAT) although, regarding CAT, the differences were not statistically significant. GSSG was more expressed in DP than in P (p<0,005) (Table 2).

Table 2. Scheffe's test results for multiple comparisons of the blood biomarkers

Group		MDA		GSH		GSSG		GSH/GSSG		CAT	
		Mean Diff	p-value	Mean Diff	p-value	Mean Diff	p-value	Mean Diff	p-value	Mean Diff	p-value
D	DP	-0.53	0.19	0.17	0.99	-0.18	0.32	0.91	0.88	-64.73	0.19
	P	0.31	0.55	-2.76*	0	0.41*	0	-4.99*	0	-110.54*	0
	C	-1.63*	0	-3.88*	0	0.69*	0	-10.78*	0	-219.14*	0
DP	D	0.53	0.19	-0.17	0.99	0.18	0.32	-0.91*	0.88	64.73	0.19
	P	0.85*	0.01	-2.93*	0.0	0.60*	0	-5.91*	0	-45.81	0.46
	C	2.17	0	-4.05*	0	0.88*	0	-11.69*	0	-154.41*	0
P	D	-0.31	0.55	2.76*	0	-0.41*	0	4.99*	0	110.54*	0
	DP	-0.85*	0.01	2.93*	0	-0.60*	0	5.91*	0	45.81	0.46
	C	1.31*	0	-1.11	0.3	0.27*	0.03	-5.78*	0	-108.6*	0
C	D	-1.63*	0	3.88*	0	-0.69*	0	10.78*	0	219.14*	0
	DP	-2.17*	0	4.05*	0	-0.88*	0	11.69*	0	154.41*	0
	P	-1.31*	0	1.11	0.3	-0.27*	0.03	5.78*	0	108.6*	0

*Significant mean diff values at the significance level <0.05. Mean Diff—mean difference between the groups.

Analyzing the mean gingival MDA and CAT values in ligatured teeth and control teeth, both biomarkers were modified in gingiva of all groups compared to the gingiva in the controls (p<0,005) (Table 3).

Table 3. Variance analysis of the gingival variables for groups D, P, DP and Control

	SS	F	p-value
MDA	3.09	10.06	0
CAT	14194.28	20.25	0

There was a correlation between MDA and CAT biomarkers in gingival tissue and blood (Figure 2).

Scheffe test revealed that gingival MDA was augmented and gingival CAT was lowered in all groups compared to Control group (p<0.005). The differences were statistically significant (Table 4). Between the pathological groups the only significant result was depicted between MDA in D and P group, where the gingival oxidative stress was more intensive in periodontitis than in diabetes. Blood and gingival CAT was more expressed in the Control group followed by P, DP and D in blood, and by D, DP and P in gingiva.

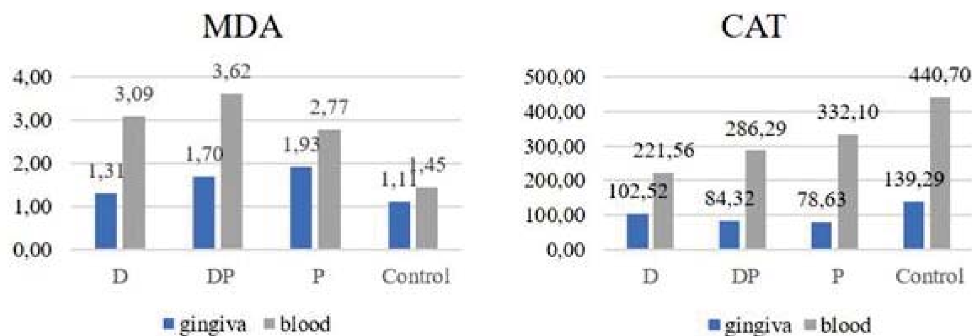

Figure 2. Mean variable evolution of oxidative stress (MDA) and antioxidant capacity biomarker (CAT) in gingiva and blood

Table 4. Scheffe's test results for multiple comparisons of the gingival biomarkers

Group		MDA		CAT	
		Mean Diff	p-value	Mean Diff	p-value
D	DP	-0.38	0.18	18.2	0.25
	P	0.61*	0.01	23.88	0.09
	C	0.19	0.68	-36.77*	0
DP	D	0.38	0.16	18.2	0.25
	P	-0.23	0.58	5.68	0.93
	C	0.58*	0.02	-54.97*	0
P	D	0.61*	0.01	-23.88	0.09
	DP	0.23	0.58	-5.68	0.93
	C	0.81*	0	-60.66*	0
C	D	-0.19	0.68	36.77*	0
	DP	-0.58*	0.02	54.97*	0
	P	-0.81*	0.02	60.66*	0
* significant mean diff values at the significance level <0.05. Mean Diff—mean difference between the groups.					

DISCUSSIONS

In our study the markers of oxidative stress and antioxidant capacity, both in blood and gingival tissue harvested from the studied animals, were significantly modified in diabetes, periodontitis and diabetes associated with periodontitis compared to controls.

For the induction of diabetes, we used STZ, an alchilant agent of DNA [23,24] that produces a rapid depletion of beta cells followed by a reduction in the secretion of insulin, hyperglycemia, a marked proinflammatory state, ROS production, oxidative stress and apoptosis [25,26].

Periodontitis was induced by placing around the molars, in the gingival sulcus, stainless-steel ligatures. The accumulation of dental plaque generates local inflammation, release of proinflammatory cytokines, polymorphonuclear cell infiltration and oxidative reactions leading to production of ROS in order to eliminate the bacteria and prevent the destruction of the periodontal tissue and bone resorption [27,28]. When the antioxidant system is overwhelmed periodontitis is installed [29,30].

The first targets of ROS are the poly-unsaturated fatty acids in the cellular membrane. Their peroxidation leads to MDA synthesis, making MDA an important indicator of the intensity of the oxidative stress [31]. In our study, blood MDA increased significantly in D, DP and P groups compared to the control group attesting the installation of the oxidative stress [32-36].

When groups D, DP and P were compared, rats with diabetes, with or without periodontitis, experienced higher circulatory MDA levels, than the periodontitis rats. The significant differences between DP and P groups could be explain as a summation of local oxidative stress implicated in the etiopathogenesis of periodontitis that is eventually manifesting at a circulatory level [27, 28] and a general oxidative stress present in the context of diabetes [7]. Our results support the theory according to which diabetes when associated with an increased lipid peroxidation could aggravate an inflammatory microbian process such as periodontitis [33]. Circulatory MDA, therefore could be considered an indicator of the association of periodontitis and diabetes.

Lowered levels of the antioxidant indicators GSH, GSSG, GSH/GSSG and CAT were corelated with the MDA levels in the tested groups compared to controls. In the rats with diabetes, DP and D groups, the antioxidant depletion was significantly lowered than in P group as the systemic oxidative stress is corelated with a depletion in the organisms antioxidative pool in the attempt of establishing the oxidants-antioxidants homeostatic equilibrium [4,37-39].

At a local, gingival level, MDA and CAT were significantly different in P and DP groups compared with the control animals indicating an augmentation of oxidative stress and a depletion of the antioxidant capacity in periodontitis and periodontitis associated with diabetes [8,40].

At the D, DP and P comparison, in periodontitis groups, MDA was found more expressed. Significant differences were depicted between D and P groups where local MDA was higher in periodontitis ($p<0,005$) [41].

Blood and gingival CAT values were significantly decreased in the tested groups compared to the control group ($p<0,05$) as a result of the organisms attempt to counteract the oxidative stress [8,40].

Between the tested groups we obtained lower values of CAT in D, DP followed by P when assayed from blood and P, DP and D when assayed from gingival tissue [41]. More significant differences could result after a longer period of time of periodontitis and diabetes evolution leading to a higher accumulation of local ROS and increased local oxidative stress.

CONCLUSIONS

Oxidative stress is implicated in the pathogenesis of periodontitis and periodontitis associated with diabetes, attested by increased circulatory and local MDA levels and reduced circulatory levels of GSH, GSSG, GSH/GSSG and CAT, and locally lowered CAT levels.

Significantly increased blood MDA and decreased blood GSH, GSSG and GSH/GSSG in periodontitis with diabetes when compared to periodontitis, indicate these oxidative stress biomarkers as potential screening tools in estimating the risk of the periodontitis and diabetes association.

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Institutional Review Board Statement: The study was approved by the Ethical Committee on Animal Welfare of "Iuliu Hațieganu" University of Medicine and Pharmacy nr. 172/13 June 2019, in accordance with the Guidelines on the Care and Use of Animals for Scientific Purposes, National Advisory Committee for Laboratory Animal Research, 2004.

Conflicts of Interest: The authors declare no conflict of interest.

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