Original Article

Level of Oxidative Stress Markers in Peri-Implant Crevicular Fluid and Their Correlation with Clinical Parameters

Mahvash Mousavi Jazi[1], Hamid Reza Sadeghi Pour Rodsari[2], Faezeh Mirmiran[3]✉

[1]Assistant Professor, Department of Periodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[2]Professor, Department of Physiology, Tehran University of Medical Sciences, Tehran, Iran
[3]Assistant Professor, Department of Periodontics, School of Dentistry, Qazvin University of Medical Sciences, Qazvin, Iran

Abstract

Objectives: Increased levels of oxidative stress markers in periodontitis have been reported by recent studies. Malondialdehyde (MDA) and superoxide dismutase (SOD) are both increased during oxidative stress. The aim of this study was to detect and measure the level of oxidative stress markers in peri-implant crevicular fluid (PICF). Their correlation with peri-implant clinical parameters was investigated as well.

Materials and Methods: PICF samples of 50 dental implants were collected in 31 patients. Peri-implant clinical parameters including probing pocket depth (PPD), gingival index (GI) and bleeding on probing (BOP) were recorded. Levels of oxidative stress markers including MDA, SOD and total antioxidant capacity (TAC) in PICF were determined.

Results: Twenty four implants showed signs of inflammation and 26 implants had healthy peri-implant tissues. MDA and TAC were detected in all samples, but SOD was not detected around 31 implants. The differences between the two groups with respect to the levels of MDA, TAC and SOD in PICF were not statistically significant ($P > 0.05$). In addition, significant correlations were observed between PPD and TAC and MDA level ($P<0.05$).

Conclusion: Significant correlations exist between PPD and level of MDA and TAC. Moreover, level of oxidative stress markers (MDA, SOD and TAC) in PICF does not significantly change in peri-implantitis compared to healthy implants. Measuring these markers in PICF does not seem to be helpful for discrimination of peri-implant health and disease status.

Keywords: Dental Implants; Oxidative Stress; Antioxidants

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✉ Corresponding author:
F. Mirmiran, Department of Periodontics, Qazvin University of Medical Sciences, Qazvin, Iran

Mirmiranf@yahoo.com

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INTRODUCTION

Endosseous dental implantation for replacement of missing teeth is an important component of modern dentistry. Monitoring the clinical status of peri-implant tissues is critical for long-term success of dental implants [1]. According to the dental literature, various clinical parameters (i.e. PPD, GI and plaque index) are used for this purpose [1-3]. In addition, it appears that analysis of the peri-implant inflammatory process on the molecular level may have a diagnostic potential for discrimination of peri-implant health/ disease status [1,4]. PICF is an osmotically mediated transudate/inflammatory exudate around dental implants and analysis of this fluid may help

early detection of metabolic and biochemical changes not easily perceivable [5].

Peri-implant conditions are due to the inflammation of the peri-implant tissues and are divided into two groups of peri-implant mucositis (reversible inflammation of the peri-implant mucosa without loss of supporting bone) and peri-implantitis (inflammation of the soft tissue around implant associated with bone loss) [6]. Similar to periodontal disease, peri-implant diseases are multifactorial; however, bacterial pathogens in conjunction with the host response further contribute to the tissue destruction around implants [7].

Production of reactive oxygen species (ROS) is an integral feature of normal cellular metabolism. An excess of ROS can cause oxidative stress and damage critical biomolecules resulting in adverse biological effects [8]. ROS can induce lipid peroxidation (LPO), with subsequent effects on cells. ROS-related tissue destruction can be measured using the final products of LPO, such as MDA, which is the most commonly studied product of polyunsaturated fatty acid peroxidation [9,10].

The host protects itself from ROS and inhibits the associated tissue destruction by releasing antioxidants [11]. SOD is an antioxidant enzyme that acts against superoxide, an oxygen radical released in inflammatory pathways, and causes connective tissue breakdown [12].

Several studies have demonstrated that oxidative stress is an important factor in pathogenesis of periodontitis [12-14].

Detection of ROS and oxidation products in the crevicular fluid and the imbalance in the oxidant/antioxidant activity in the periodontal pocket suggest the significant role for ROS in periodontal tissue destruction [15].

Although the role of ROS in periodontal disease has been extensively studied, there is a gap of information about the role of oxidative stress in peri-implant disease. To the best of our knowledge, no previous study has evaluated the level of oxidative stress markers in PICF.

The purpose of this study was to detect and measure the level of oxidative stress markers (MDA, SOD and TAC) in PICF and also evaluate their correlation with peri-implant clinical parameters.

MATERIALS AND METHODS

This study was conducted on 31 individuals (23 females and 8 males), selected from a group of patients who received dental implants at the Implantology Department of the School of Dentistry, Tehran University of Medical Sciences from March to September 2012.

The inclusion criteria were: 1) Presence of at least one endosseous dental implant with an appropriate prosthesis in partially edentulous patients, 2) Dental implant had to be in function for at least 6 months.

The exclusion criteria were: 1) History of medical conditions that required antibiotic prophylaxis, 2) Presence of active periodontal disease, 3) Use of antibiotic or anti-inflammatory medications in the past 3 months, 4) Pregnancy, 5) Use of vitamin supplements, 6) Smoking. Prior to the onset of this study, all patients signed written informed consent forms.

Based on the results of PPD, GI and BOP, the examined peri-implant sites were divided into two groups of healthy implants (PPD $\leq$ 3mm, GI = 0 and BOP = 0) and diseased implants (PPD $\geq$ 4mm, GI $\geq$1 or BOP = 1). The criteria have been thoroughly described in previous studies [15-18].

Clinical measurements

The clinical examinations were performed after PICF sampling. All procedures were carried out by a single examiner. The clinical examinations included assessment of peri-implant PPD, GI [19] and BOP [20].

All measurements were made using a Williams probe with markings indicating 1mm increments. The measurements were made at six areas around each implant. Maximum value of PPD for each implant was recorded.

PICF sampling

Sites selected for PICF sampling were isolated with cotton rolls. Sample collection was performed within 30 seconds with standardized paper strips (Whatman Industries, Dartford, Kent, UK) from the buccal and lingual/palatal crevices around each implant. Then, PICF samples were placed in sterile Eppendorf tubes containing 300 µL of phosphate buffered saline (PBS). Samples were eluted for 30 minutes at room temperature before removing the Periopaper strips and then stored at -70° C until analysis.

Analysis of MDA level

The MDA level in PICF was determined by the method described by Esterbauer and Cheeseman [21] based on its reaction with thiobarbituric acid (TBA) at 90–100°C and measurement of the absorbance at 532 nm. MDA reacts with TBA and produces a pink pigment which has maximum absorption at 532 nm. The value of each sample was obtained from the standard curve and expressed as nmol/mL.

Analysis of SOD activity

SOD activity was determined by the method described by Paoletti and Mocali [22]. In this method, superoxide anions are generated from oxygen molecules in the presence of $EDTAMnCl_2$ and mercaptoethanol. NAD (P) H oxidation is linked to the availability of superoxide anions in the medium. As soon as SOD is added to the assay mixture, it inhibits nucleotide oxidation. Therefore, at high concentration of the enzyme, the absorbance at 340 nm remains unchanged. For this purpose, PICF samples were homogenized in 300 µL of phosphate buffer. After 30 minutes, the mixture was centrifuged at 4200 rpm; 400 µL of the supernatant was added to 1 mL of phosphate buffer, and was then inserted in dialysis tubes inside the phosphate buffer for 15-18 hours (4°C). The following solutions were subsequently added to the cuvette: 0.8 mL of triethanolamine-diethanolamine-HCl buffer, 40 µL of NADPH solutions, 25 µL of $EDTAMnCl_2$ and 100 µL of different samples. In the 5th minute, mercaptoethanol was also added. Absorbance changes were detected at 340 nm. The SOD activity was determined according to the manufacturer's formula and results were reported in u/mL.

Analysis of TAC

TAC of PICF samples was measured using the antioxidant assay kit (Cayman Chemical Company, MI, USA).

The reaction was based on the ability of aqueous and lipid antioxidants to inhibit the oxidation of the 2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) to ABTS+. The capacity of the antioxidants to prevent ABTS oxidation was then compared with that of standard Trolox, a water-soluble tocopherol analogue. Absorbance was measured at 405 nm according to the manufacturer`s instructions using Tecan Sunrise microplate reader (Tecan Austria GmbH, Grödig, Austria). The unit of measurement was g/dL.

Statistical analysis

Statistical analysis was performed using SPSS version 11.5. All measurements were made and reported as mean ± standard deviation. Mann-Whitney u-test was performed to compare the level of oxidative stress markers between the two groups.

Also, for assessment of correlation among clinical parameters and oxidative stress levels, Spearman correlation test was applied. $P < 0.05$ was considered significant.

RESULTS

Clinical findings: In this study, 50 dental implants were evaluated; 24 implants showed signs of inflammation and 26 implants had healthy peri-implant tissues. Table 1 shows the clinical data for the healthy and diseased peri-implant states.

Laboratory findings: The mean values of MDA, SOD, and TAC in healthy and diseased peri-implant states are shown in Table 2.
With respect to the levels of MDA, SOD and TAC of PICF, differences between the two groups were not statistically significant (P=0.195, P=0.09 and P=0.169, respectively). It is noteworthy that SOD was detected in PICF of 19 (38%) implants only and it was not detected in PICF of 31 implants.

Correlations: Significant correlations were observed between PPD and TAC and also between PPD and MDA level. Correlations between other clinical parameters and level of oxidative stress markers were not significant.
The correlations among clinical parameters and level of MDA, SOD and TAC of PICF are presented in Table 3. In addition, there were statistically significant correlations between TAC and SOD activity (P=0.009, r=0.365) and also between MDA and TAC (P=0, r=0.554).

DISCUSSION

This is the first study assessing the level of oxidative stress markers (MDA, SOD and TAC) in PICF and the results of this investigation revealed the presence of these markers in PICF. In our study, level of MDA was higher in PICF of diseased compared to healthy peri-implant conditions, but this difference was not statistically significant.

Table 1. Clinical data of each group

PPD (mm)	Healthy	Diseased
	1.8 ± 0.7	**3.6 ± 1.2**
% sites with gingival inflammation (GI ≥ 1)	0	79.2
% sites with bleeding on probing (BOP=1)	0	48

Table 2. The mean level of MDA, SOD and TAC in the two groups

	Healthy	Diseased
TAC (g/dL)	1.33± 0.056	1.35±0.0466
SOD (u/mL)	0.003±0.006	0.006±0.008
MDA nmol/mL	1.34±0.662	1.46±0.489

TAC: Total antioxidant capacity, SOD: Superoxide dismutase, MDA: Malondialdehyde.

Table 3. The measures of pearson correlation coefficients between clinical parameters and level of oxidative stress markers

Parameters		TAC	SOD	MDA
GI	r	0.216	0.118	0.211
	P	0.132	0.413	0.141
PPD	r	0.357	0.091	0.455
	P	0.011	0.529	0.001
BOP	r	0.242	0.196	0.185
	p	0.090	0.172	0.198

PPD: Probing pocket depth, GI: Gingival index, BOP: Bleeding on probing, TAC: Total antioxidant capacity, SOD: Superoxide dismutase, MDA: Malondialdehyde, r: correlation coefficient, p: significance level

Several studies have analysed the level of MDA in GCF of patients with periodontal disease.They found that level of MDA was significantly higher in GCF of periodontally compromised patients than periodontally healthy group [13,23,24].

In our study, no statistically significant difference was found between the two groups with respect to the level of SOD activity in PICF. This finding is in agreement with the results of a study carried out by Akalin et al, [12] who reported no significant difference in level of SOD in GCF of chronic periodontitis patients and control group, suggesting poor activity of SOD in GCF of periodontal patients. The other issue that must be taken into consideration is that in our study, SOD was detected only in PICF of 19 implants out of a total of 50. SOD is mainly found in cells and tissues and has only minor activity in extracellular fluids [25,26]. This may be one of the possible reasons for the weak activity of SOD in PICF in the current study. Another possibility may be that increased generation of ROS and oxidative damage may have supressed SOD production in PICF [27].

In the current study, no significant difference was observed in TAC of PICF between the groups.

This result is inconsistent with the findings of a study conducted by Liskmann et al, [15] who assessed the antioxidant capacity of saliva in peri-implant health and disease states and reported that in patients with peri-implant disease, total antioxidant status of saliva and concentration of main salivary antioxidants (uric acid and ascorbate) decreased significantly. In contrast to our study, fully edentulous patients with implant supported overdentures were included in the study by Liskmann et al [15]. We chose PICF for sampling instead of saliva and serum, because the reduction in antioxidant capacity of PICF is likely to be of greater significance in peri-implant disease than the systemic changes that occur in serum and saliva. Antioxidants in PICF may play an important role in dampening down peri-implant inflammatory processes [15]. Accurate comparison between samples requires standardized sample collection. This is achieved by sampling all sites for the same length of time [28]. Thus, we preferred to standardize the collection time rather than the PICF volume to analyse the activity of biomarkers. Also, we expressed total amounts instead of concentrations for data presentation. The findings of Smith et al. [29] indicated that total amounts of GCF (and PICF) components per site were more closely associated with the activity of periodontal disease than concentrations. Furthermore, in our study, there were significant correlations between PPD and level of MDA and TAC of PICF. Similarly, Akalin et al. [13] found strong correlations between periodontal parameters and MDA and total antioxidant status of GCF.

CONCLUSION

According to the results of this study, significant correlations exist between PPD and level of MDA and TAC. Moreover, level of oxidative stress markers (MDA, SOD, TAC) in PICF does not significantly change in peri-implant conditions compared to healthy status. Therefore, it seems that the measurement of these markers in PICF is not helpful to differentiate between the peri-implant health and disease conditions. The results of this investigation should be interpreted with caution due to its cross-sectional design. Longitudinal studies with a larger sample size are required to draw a definite conclusion.

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