Oxidative stress and peri-implantitis: The role of oxidants and antioxidants

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Abstract

Background: Peri-implantitis is the main cause of implant failure and is associated with augmented oxidative stress or tissue destruction. In this study, it was aimed to investigate the oxidant-antioxidant balance parameters in individuals with peri-implantitis, considering the clinical findings and the control group.

Methods: Peri-implantitis (n=30) and healthy (n=30) individuals participated in the study. Peri-implant clinical parameters, including probing depth (PD), gingival index (GI), plaque index (PI), bleeding on probing (BoP), and keratinized mucosa width (KMW), were recorded. The levels of total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI), and arylesterase (ARE) in saliva were examined. To identify the relationship between oxidative stress biomarkers and clinical parameters was used Spearman’s correlation coefficient.

Results: TOC values were higher in peri-implantitis, and they correlated with BoP, GI, PD, and PI (P = 0.004, r = 0.370; P = 0.010, r = 0.328; P = 0.038, r = 0.268; P = 0.007, r = 0.342, respectively). TAC values were higher in healthy and correlated with PI, BoP, and GI (P = 0.021, r = -0.297; P = 0.015, r = -0.273; P = 0.012, r = -0.321, respectively). OSI showed a negative correlation with the KMW (mm) (P = 0.046, r = -0.259).

Conclusion: Increased TOC and decreased TAC and ARE activity could be predictors of peri-implantitis development. Adequate KMW is important in the production of antioxidants.

Keywords: Dental implants, Peri-implantitis, Oxidative stress, Saliva, Arylesterase

Introduction

Peri-implantitis is characterized by chronic plaque-related pathology in the peri-implant mucosa leading to supportive bone loss.\textsuperscript{1} Clinical findings of peri-implantitis are bleeding during probing, suppuration, increased pocket depth on clinical examination, and bone loss on radiographic examination.\textsuperscript{2} Epidemiological studies highlight some risk factors associated with peri-implantitis, including poor oral hygiene, insufficient keratinized mucosa width (KMW), genetic predisposition, smoking, and alcohol consumption.\textsuperscript{1,2} To date, peri-implantitis has been evaluated based on several clinical parameters such as plaque index (PI), gingival index (GI), probing depth (PD), and bleeding on probing (BoP). New biochemical parameters associated with oxidative stress have gained importance in evaluating the effect of antioxidants and oxidants on dental implant survival.\textsuperscript{3} Oxidative stress variables of the environment can play a very effective role in the activation of biological pathways during wound healing and osseointegration following implant treatment.\textsuperscript{3} In line with this, total oxidant capacity (TOC) is an important candidate for the analysis of the total concentration of oxidant molecules. Similarly, total antioxidant capacity (TAC) is responsible for balancing the oxidative stress burden.\textsuperscript{3}

TOC’s unique structure and chemical composition make it possible to measure the maximal amount of all enzymatic and non-enzymatic antioxidants.\textsuperscript{1} In addition, the ratio of TOC to TAC, which is used to calculate the oxidative stress index (OSI), is a very important indicator in this dynamic oxidative network.\textsuperscript{5}

At the heart of this system, paraoxonase 1 (PON1) and arylesterase (ARE) occupy an important place in this endogenous antioxidant system.\textsuperscript{5} PON1, which is mainly produced in the liver and released into the blood. There are three known enzymatic subunits: PON, ARE, and dyazoxonase. It has now become clear that increased oxidative stress causes a decrease in PON1 activity.\textsuperscript{7,8} Besides, ARE is important in the process of oxidation of lipid and glycose metabolites, and unlike PON1, it has no...
polymorphic genetic alteration. Stable crosstalk between ARE activity and the PON1 gene has enormous potential in controlling several inflammatory and autoimmune diseases such as metabolic syndrome, diabetes mellitus, and cardiovascular diseases. However, the evaluation of these antioxidant biomarkers in the saliva of patients with peri-implantitis is limited. Therefore, we aimed to investigate TAC, TOC, OSI, and ARE activity in saliva samples of participants with dental implants (peri-implantitis and healthy groups) and appraise the correlation of these levels with the clinical parameters.

Methods

Patient selection criteria
Sixty participants who had one or more dental implants participated in this work. All implants had been in function for 3 years, and the participants were divided into two groups: 30 peri-implantitis and 30 healthy. Ethics committee approval was obtained for the study (2020-228) and all the participants signed a consent form. The study was conducted in February-April 2021. All individuals underwent a detailed and standardized clinical examination, and they were diagnosed according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Accordingly, increased PD (≥ 6 mm), presence of bone loss, bleeding, and/or suppuration on gentle probing were examined. The control group consisted of patients with dental implants without a history or clinical manifestations of peri-implantitis. This group consisted of individuals with no bone loss beyond crestal bone level changes and no bleeding and/or suppuration on mild probing. Patients with a history of systemic disorders, drug administration during the past three months, bisphosphonates, monoclonal antibodies, high-dosage corticosteroid therapy, or radiotherapy of the cervicofacial area were excluded from the study.

Clinical assessment
Parameters such as PD, GI, BoP, and KMW were determined using the periodontal probe (Williams: Hu-Friedy, Chicago, IL, USA), and the data were recorded. Calibration was performed on ten peri-implantitis patient samples before the study. Assessment of the probing PD was performed in two separate sessions 48 hours apart, and the total kappa value of 0.92 which calculates the reliability of each examiner, indicated an acceptable degree of agreement.

Saliva sampling/analysis of oxidative stress biomarkers
For the analysis of the parameters, a well-ventilated and well-lit clinical environment was chosen for the collection of unstimulated saliva samples. Sample collection in patients was performed between 9 and 10 AM to avoid circadian rhythm changes. Individuals were instructed not to eat or drink anything for at least 1 hour before the unstimulated saliva sample collection. A total amount of 3 ml of saliva was collected from each participant and stored at -80°C until biochemical analysis. TAC (mmol/L) and TOC (μmol/L) and ARE activity (U/L) were analyzed spectrophotometrically. OSI is a crucial marker in this dynamic oxidative network, and the analysis of OSI was made using this formula:

\[ \text{OSI} = \frac{TAC}{TAC + TOC} \]

Statistical analysis
We used the Kolmogorov-Smirnov test to evaluate whether the obtained data in this study were normally distributed. To assess the differences between these groups, we used the student t test for normally distributed variables (GI, PI, PD, KMW, and age), and Mann-Whitney U test for variables without normal distribution (TAC, TOC, OSI, and ARE). Quantitative results were interpreted with the chi-square test and shown as mean ± standard deviation (SD). Spearman’s correlation coefficient was used to identify the relationship between oxidative stress biomarkers and clinical parameters. Statistical significance was set as \( P < 0.05 \).

Results

Clinical findings
Our study included 60 patients (35 males and 25 females) with dental implants, 30 of whom had peri-implantitis (15 males and 15 females with a mean age of 51.86 ± 11.96) and 30 were healthy (20 males and 10 females with a mean age of 46.76 ± 8.83). Clinical data are shown in Table 1. A total of 104 dental implants were evaluated in this study, 49 of which showed signs of inflammation and 55 had healthy peri-implant tissues. The mean follow-up period of the participants was 3 years. In both groups, dental implants were mostly located in the posterior mandible (Table 1). Student t test for normally distributed variables and data did not find statistical significance.

Laboratory findings
The mean values of TAC, TOC, ARE, and OSI in peri-implantitis and healthy groups are given in Table 2. The differences in TAC, TOC, OSI, and ARE levels were statistically significant between the groups (\( P = 0.008, P = 0.002, P < 0.001, P = 0.002 \), respectively, Table 2).

Table 1. Descriptive statistics between the groups

<table>
<thead>
<tr>
<th>Age (mean ± SD)</th>
<th>Peri-implantitis (n = 30)</th>
<th>Healthy (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51.86 ± 11.96</td>
<td>46.76 ± 8.83</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>15.15</td>
<td>10.20</td>
</tr>
<tr>
<td>Number of dental implants</td>
<td>49</td>
<td>55</td>
</tr>
<tr>
<td>Maxilla/Mandible (n)</td>
<td>20.29</td>
<td>24.31</td>
</tr>
<tr>
<td>Anterior/Posterior (n)</td>
<td>18.31</td>
<td>26.29</td>
</tr>
<tr>
<td>SD: standard deviation.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Besides, statistically significant differences were observed in all parameters (GI, PI, PD, and KMW) \((P < 0.001, \text{Table 3})\). BoP was a clinical parameter observed predominantly in the peri-implantitis group at a rate of 80% \((P < 0.001, \text{Table 3})\).

**Correlations**

The correlations among clinical parameters and values of TAC, TOC, ARE, and OSI are presented in Table 4. It was observed that the increase in TOC triggered edema and inflammation of the peri-implant tissues concerning GI \((P = 0.010, r = 0.328)\). BoP, which reflects histological, clinical, and bacteriological changes, is important in detecting infection in the early period and was associated with increased TOC \((P = 0.004, r = 0.370)\). It was observed that plaque accumulation, which is one of the etiological factors of chronic gingivitis, caused an increase in TOC \((P = 0.007, r = 0.342)\). PD, which is effective in showing the degree of inflammation in peri-implant tissues, had a significant correlation with TOC \((P = 0.038, r = 0.268)\). The TAC level correlated negatively with PI, BoP, and GI, supporting the conclusion that peri-implantitis may be rare around implants when proper plaque control is maintained \((P = 0.021, r = -0.297; P = 0.035, r = -0.273; P = 0.012, r = -0.321, \text{respectively, Table 4})\). Besides, as KMW (mm) decreased, OSI increased \((P = 0.046, r = -0.259)\). Figure 1 shows that the simultaneous increase in the oxidants and PD was accompanied by decreased TAC values. Similarly, the increase in oxidants was correlated with BoP (Figure 2). It was observed that antioxidants were induced with the increase in KMW (mm) (Figure 3).

**Discussion**

Endosseous dental implantation to replace missing teeth is the mainstay of modern dentistry. However, there are some major potential complications of dental implants, including delayed wound healing, rejection of the implant, loss of bone density, and peri-implantitis. Early intervention for these complications is important for implant survival.

Oxidative stress may reduce secretion from salivary glands and change their biochemical composition.18 The connection between salivary antioxidant levels and pathological conditions indicates the importance of antioxidant defense in the periodontal tissue.19 As known, saliva is the first defense against oxidative stress, and its assessment is an easy method to evaluate the oxidative microenvironment in oral diseases.20,21 In our study, we evaluated the correlation between several oxidative stress-related biomarkers (TAC, TOC, OSI, and ARE) and clinical parameters (PD, GI, PI, and KMW) in patients with peri-implantitis and in controls. Research recently has emphasized the role of oxidants in peri-implantitis.21 Mijiritzky et al attributed the hyperinflammatory response required for peri-implantitis to the redox imbalance due to the increase in oxidant levels.22 In our samples, we found a large amount of TOC in peri-implantitis \((41.94 \pm 32.81 \mu\text{mol/L})\) compared to the controls \((21.58 \pm 28.87 \mu\text{mol/L})\). Also, our data showed the increase in TOC was associated with PI, GI, PD, and BoP. Mousavi Jazi et al reported that increased malondialdehyde values in the gingival crevicular fluid were associated with deep pocket depth \((P = 0.001, r = 0.455)\).2 These results support the presence of this marker in peri-implantitis. In a previous study, malondialdehyde was higher in peri-implantitis \((0.52 \pm 0.37 \mu\text{M/L})\) compared to healthy implants \((0.40 \pm 0.16 \mu\text{M/L})\).21 In addition, higher myeloperoxidase concentrations were found in the peri-implantitis group \((12.32 \pm 2.17 \text{ng/mL})\) compared to healthy implants \((11.54 \pm 2.80 \text{ng/mL})\). Evidence supports the conclusion.

**Table 2.** The mean values of TAC, TOC, OSI and ARE in two groups

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Peri-implantitis</th>
<th>Healthy</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC ((\text{mean} \pm \text{SD}))</td>
<td>0.70 ± 0.39</td>
<td>1.07 ± 0.57</td>
<td>0.008*</td>
</tr>
<tr>
<td>TOC ((\text{mean} \pm \text{SD}))</td>
<td>41.94 ± 32.81</td>
<td>21.58 ± 28.87</td>
<td>0.002*</td>
</tr>
<tr>
<td>OSI ((\text{mean} \pm \text{SD}))</td>
<td>6.83 ± 4.85</td>
<td>2.45 ± 4.70</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ARE ((\text{mean} \pm \text{SD}))</td>
<td>320.76 ± 7.42</td>
<td>324.20 ± 19.61</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

TAC: total antioxidant capacity, TOC: total oxidant capacity, OSI: oxidative stress index, ARE: arylesterase, SD: standard deviation.

*\(P\) values \(<0.05\) were considered statistically significant.

**Table 3.** Comparison of clinical parameters between the groups

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Peri-implantitis</th>
<th>Healthy</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI ((\text{mean} \pm \text{SD}))</td>
<td>1.90 ± 0.27</td>
<td>0.00 ± 0.00</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PI ((\text{mean} \pm \text{SD}))</td>
<td>1.63 ± 0.54</td>
<td>0.13 ± 0.28</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PD ((\text{mean} \pm \text{SD}))</td>
<td>4.90 ± 0.80</td>
<td>2.93 ± 0.41</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BoP (%)</td>
<td>80%</td>
<td>0%</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>KMW ((\text{mean} \pm \text{SD}))</td>
<td>1.55 ± 0.83</td>
<td>2.59 ± 0.67</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>


*\(P\) values \(<0.05\) were considered statistically significant.

**Table 4.** Correlations between clinical parameters and level of oxidative stress biomarkers

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>TAC</th>
<th>TOC</th>
<th>OSI</th>
<th>ARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>(r = -0.321)</td>
<td>0.012</td>
<td>0.010</td>
<td>0.001</td>
</tr>
<tr>
<td>PI</td>
<td>(r = -0.297)</td>
<td>0.021</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PD</td>
<td>(r = -0.198)</td>
<td>0.130</td>
<td>0.038</td>
<td>0.012</td>
</tr>
<tr>
<td>BoP</td>
<td>(r = -0.273)</td>
<td>0.035</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>KMW</td>
<td>(r = 0.164)</td>
<td>-0.194</td>
<td>-0.259</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Figure 1. Correlation between PD and oxidative stress biomarkers (A) Correlation between PD and TAS, (B) Correlation between PD and TOS, (C) Correlation between PD and OSI, (D) Correlation between PD and ARE

Figure 2. Correlation between BoP and oxidative stress biomarkers (A) Correlation between BoP and TAS, (B) Correlation between BoP and TOS, (C) Correlation between BoP and OSI, (D) Correlation between BoP and ARE
that oxidants can play a critical role in the etiology of peri-implantitis and trigger the progression of bone loss.\textsuperscript{22} TAC, which is the sum of both enzymatic and non-enzymatic antioxidants, is involved in the suppression of inflammation and periodontal diseases.\textsuperscript{23} Decreased antioxidant capacity and augmented oxidative stress biomarkers have been reported in patients with periodontitis.\textsuperscript{24} A lower amount of TAC was observed in our samples in patients with peri-implantitis (0.70 ± 0.39 mmol/L) compared to the controls (1.07 ± 0.57 mmol/L). Also, our data showed the decrease in TAC was associated with BoP, PI, and GI. Recently, researchers have noted that the increase in oxidants is a remarkable factor that increases the destruction of peri-implant tissues. They have assessed values of various antioxidants in saliva to identify differences between the saliva in healthy and peri-implantitis. They found that TAC was 0.41 ± 0.10 in the control group and 0.19 ± 0.07 in the peri-implantitis group.\textsuperscript{25} The study of Shapira et al\textsuperscript{26} found that the expression of oxidants in gingival tissues was triggered by bacterial stimulation while there was no change in antioxidant capacity. This is completely consistent with the result that an increase in antioxidant levels can be achieved after non-surgical treatment in chronic periodontitis.\textsuperscript{27} The results of another work proposed that nanoparticle antioxidants can suppress bacterial inflammation and alveolar bone resorption by eliminating ROS and inhibiting osteoclast growth.\textsuperscript{28}

The effect of KMW on the frequency of peri-implant diseases is a heavily debated subject in the literature. Recently researchers have stated that KMW plays a null role in preventing biological complications in highly compliant patients, highlighting the importance of frequent peri-implant care.\textsuperscript{29} Moreover, it is ported that the presence of ≥ 2 mm keratinized mucosa is beneficial for long-term outcomes in incompatible patients.\textsuperscript{30,31} Schrott et al\textsuperscript{32} reported that a minimum of 2 mm of KMW is essential for decreased plaque accumulation and bleeding tendencies in patients with good oral hygiene. Monje and Blasi showed that PD, BI, PI, or marginal bone loss increased in implants with KMW less than 2 mm.\textsuperscript{31} Our data showed that an increase in OSI was detected as KMW (mm) decreased (\(P = 0.046, r = -0.259\)). Ueno et al found that reduced KMW (< 2 mm) was associated with PD, BoP, and the presence of plaque.\textsuperscript{33} The results of our study confirm that adequate KMW positively affects oral hygiene procedures and prevents severe peri-implant mucositis and high gingival/plaque index levels.\textsuperscript{34}

Oxidative stress has several impacts on intracellular metabolic reactions such as lipid peroxidation, protein oxidation, and genomic damage. In these processes, several antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase are known to be effective. Otherwise, these important enzymatic antioxidants, PON1 has antioxidative properties as well.\textsuperscript{35}
PON1 is an enzyme with three enzymatic subunits, which are PON, ARE, and dyazoxonase. Among these proteins, ARE has a critical role in the process of oxidation of lipid and glucose metabolites.\textsuperscript{5} The ARE activity of PON1 regulates many cellular functions, including lipid peroxidation, ROS accumulation, DNA methylation, and glucose and blood pressure homeostasis.\textsuperscript{11,36}

Recent evidence has shown lower serum PON1 and ARE activity in osteomyelitis compared to healthy.\textsuperscript{27} Also, in sepsis showed significantly lower values of PON \((85.1 \pm 16.6 \text{ U/L})\) and ARE activity \((114.9 \pm 15.2 \text{ U/L})\) than healthy controls.\textsuperscript{38} This study suggests that these enzyme activities can be used to predict outcomes in patients with sepsis. In our study, ARE activity in saliva was \(320.76 \pm 7.42 \text{ U/L}\) in peri-implantitis and \(324.20 \pm 19.61 \text{ U/L}\) in the control group. This is the first report showing lower ARE activity with higher oxidant parameters in peri-implantitis. As a result, decreased ARE activity and the absence of oxidant-antioxidant homeostasis may disrupt peri-implant tissue integrity. However, further clinical work is needed to clarify the possible pathophysiological role of the biomarkers.

Although there is no definite treatment approach, the treatment of peri-implantitis consists of antibiotics, mechanical lavage, and surgical approaches. Identifying new approaches to prevent peri-implantitis is essential for implant success.

**Strengths and Limitations**

The major limitation of the study is that it is cross-sectional. In addition, salivary fluid is affected by many fluids. These data can be supplemented with gingival cavities fluid and serum samples. Saliva can also be affected by systemic and oral physiological-biochemical conditions. Strengths: The effectiveness of oxidative stress on peri-implantitis has been observed from a broad perspective and this has been supported by ARE.

**Conclusion**

This study showed that oxidative stress and inflammatory microenvironment have a more relevant effect on dental implant survival than previously thought. On this basis, further studies are recommended to identify and validate a wide range of oxidants that can prove useful in the early detection of peri-implantitis.

**Authors’ Contribution**

Conceptionalization: Gülbahtar Ustaoglu, Deniz Yaman, Emre Avci.
Data investigation: Gülbahtar Ustaoglu, Deniz Yaman, Emre Avci.
Formal analysis: Deniz Yaman, Emre Avci.
Methodology: Gülbahtar Ustaoglu, Deniz Yaman, Emre Avci.
Project administration: Gülbahtar Ustaoglu, Deniz Yaman, Emre Avci.
Supervision: Gülbahtar Ustaoglu, Deniz Yaman, Emre Avci.
Software: Deniz Yaman, Emre Avci.
Resources: Gülbahtar Ustaoglu, Deniz Yaman.
Validation: Deniz Yaman, Emre Avci.


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