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# **Evaluation of Whole Saliva Antioxidant Capacity in Patients with Periodontal Diseases**

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## **Abstract**

Background and purpose: Periodontitis is the inflammation of the supporting tissues of teeth. In this disease, overproduction of free radicals by polymorphonuclears (PMNs) will result in the imbalance between saliva's oxidants and antioxidants. Therefore, due to insufficient levels of antioxidants, free radicals can start chain reactions that eventually will lead to destruction of periodontal tissues. The purpose of this research is to compare the amount of whole saliva's antioxidant capacity in people with major periodontitis with that of healthy people. Methods and materials: In this case-control study, the amount of antioxidants capacity and saliva antioxidants were measured in 43 cases with periodontitis, as well as in 43 healthy people (controls); and the measurements were compared between the two groups. The amount of antioxidants capacity and their saliva antioxidants capacity was measured by FRAP (ferric reducing ability of plasma) and with the help of a Spectrophotometer. Findings: Average and Standard deviation of saliva's antioxidants was  $184.25 \pm 63.209$  in subject group and  $399.419 \pm 90.298$  micro moles in control group. Using the T-TEST revealed that the difference in capacity of whole saliva's antioxidants in two groups is significant. Conclusion: whole saliva's antioxidants capacity is less among patients with periodontitis compared to control group.

### **Keywords**

Anti-Oxidant, Chronic Periodontitis, Saliva

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### 1. Introduction

Periodontitis is an inflammatory disease of supporting tissues of teeth caused by specific microorganisms. Activation of immune system may lead to progressive destruction of the periodontal ligament and alveolar bone including pocket formation or gingival recession or both. Bacterial products can also affect the immune system that may result in tissue damage if left untreated [1]. While reactive oxygen species (ROS) or free radicals such as  $(O_2)$  and  $H_2O_2$  release from PMNs during phagocytosiscan eliminating periodontal pathogens, they may also damage the host cells.

ROS are extremely toxic and can result in DNA LPO (lipid peroxidation), protein damage, enzyme oxidation and stimulation of monocytes and macrophages to release pre-inflammatory cytokines [1] [2], which in turn may cause oxidative stress and disruption of cell integrity [3]. Oxidative stresses could also damage connective tissues and cause bone lysis around the tooth root resulting in loosening the tooth [4].

Antioxidants are released by PMNs, and they protect the body against reactive oxygen species. Antioxidants also exist in submandibular and parotid salivary glands as a normal anti-bacterial component [5]. Antioxidant alterations in some inflammatory and pre-cancerous diseases have been assessed and confirmed [6].

While some studies have shown that antioxidants levels are changed n periodontitis [7]-[10], some other ones have failed to show this [9]. Considering contrary results of previous studies on this issue, this study was conducted to assess the level of whole saliva antioxidants patients with chronic periodontitis.

#### 2. Methods and Materials

This case control analytical research which was performed on 43 patients who presented to the oral and maxillofacial medicine department, and 43 healthy controls. Exclusion criteria were as follows: 1) taking regular medication and vitamins in the previous month; 2) history of heavy cigarette smoking in the last two years; 3) history of periodontal treatment in the last two years; 4) pregnancy. Written consent was taken from all patients and controls. After performing phase 1 of periodontal treatment (scaling and oral hygiene) 3 mL of non-stimulated saliva was collected during 5 minutes between 10 - 12 a.m. Tubes were kept in the dry ice and were sent to ErfanLabratoary with a specific code. Samples were kept in 4 degrees Celsius for 10 minutes, and then were centrifuged at 4000 rpm in minus 80 degrees Celsius. All samples were collected in the same day (same temperature and humidity) and the level of antioxidants were calculated by FRAP method (ferric reducing ability of plasma) with the use of pharmacia spectrophotometer, and ENGLAN ultropec 3000 biotech (made in McKinley Scientific). Whole plasma antioxidants were evaluated by means of Benzie et al. method [10], which is based on plasma and saliva capacity to reduce Fe<sup>+3</sup> to Fe<sup>+2</sup> in the presence of TPTZ (2,4,6,-tripyridyl-s-triazine). Fe<sup>+3</sup>-TPTZ complex is reduced under acidic conditions by a reducing agent to form Fe<sup>+2</sup>-TPTZ. The production of Fe<sup>+2</sup>-TPTZ is easily detectable and measurable because of its intense blue color with a maximum absorption at wavelength 593 nm. Therefore, the determining factor to evaluate the speed of Fe<sup>2</sup>-TPTZ complex formation and production of blue color is the reducing ability of samples. As a result, this method is able to determine every anti-oxidant component which has a reductive potential. All sample solutions for measuring whole saliva antioxidants were gathered in the same day, and all utensils were washed by 10% Nitric acid and distilled water. Then the samples were kept in minus 80 degrees Celsius until the test date. Acetate buffers were prepared by mixing 3.1 grams of sodium acetate in 16 mL of acetic acid. Then, by adding distilled water the volume was increased to 1 liter; and the solution's PH was set to 3.6. The main reactive solution was a mixture of 2.5 mL of TPTZ, 2.5 mL of iron chloride plus 25 mL of acetate buffer. During the test, temperature was set to 37 degrees Celsius. Spectrophotometer at 593 nanometer wavelength converted to zero by pouring 900 microliter of solution into the cuvette. The reaction started by adding 30 microliter of gathered saliva that was earlier centrifuged at 4 degree Celsius. Absorption was measured at the beginning of the reaction and after 8 minutes. The differences between final and initial absorptions were measured and determined using the standard curve, which were drawn by using iron sulfate. The obtained data from regression equation was a base for the statistical analyses, which was done using the SPSS ver: 16 software and T-TEST.

# 3. Findings

The mean age of case group was  $48.3 \pm 10.9$  years, and that of control group was  $38 \pm 7.27$  years.

The results show that the average and standard deviation capacity of whole saliva antioxidants was  $184.256 \pm 63.209$  micro moles per mL in case group. By using T-TEST, the decrease in capacity of whole saliva antioxidant in case group was found to be statistically significant (Table 1).

Table 1. Total antioxidant concentration in case and control groups.

variables	number	Maximum	Minimum	Mean	SD	P-value
TAO in case group	43	196	516	399.42	90.3	P < 0.001
TAO in control group	43	96	336	184.26	63.21	

### 4. Discussion

Our study showed that the amount of antioxidant in saliva of patients was lower than that of healthy control group. The results of this research were similar to that of Miricesue *et al.* study. That study showed that the amount of whole saliva's antioxidants in smokers, patients with lichen planus and periodontitis were less than healthy subjects [11]. In addition, in Novakovic *et al.* study, the reduction of antioxidant was also seen and confirmed [12].

In 2010, Kim al found out that the whole saliva antioxidants level increases after scaling [2].

In contrast, Moore *et al.* showed that there was no difference in amount and activity of antioxidants in case and healthy control groups, however, the number of patients in that study was not enough, and urea acid was considered as the most important antioxidant of saliva [13].

Chapple *et al.* study also showed that the amount of antioxidants is similar in patients with mild or severe periodontitis and healthy people. But in gingival crevicular fluid (GCF), the amount of antioxidants in case group was less than that of normal subjects. In addition, after scaling the amount of total antioxidant capacity (TAOC) in case group raised to the normal level [14].

Pathogens like *P. gingivalis* also secret their own antioxidants which enable them to resist against the killing effect of free radicals of neutrophils [15].

In saliva of healthy people, there is a balance between oxidants and antioxidants, while in that of unhealthy individuals, oxidants have a higher level that can cause oxidative stresses [4].

In periodontitis, the antioxidant system including superoxide dismutase (SOD), uric acid, ascorbic acid, Keto ferrule, myeloperoxidase, glutathione and albumin will be suppressed. SOD is one of the most important antioxidants that catalyze ions, and it protects the cells against the harmful effects of ROS. SOD also decreases significantly in the deep periodontal pocket [2].

A recent study has shown the importance of defensive role of antioxidants in saliva.

In conclusion, regarding the huge difference in the level of antioxidants between healthy individuals and patients, it is fair to conclude that production of ROS has the principal role in the initiation of the periodontal disease. Early diagnosis of periodontal disease is the best way to control the disease.

More researches are needed to further clarify the results of this study.

### 5. Conclusion

According to the results of this study, the average amount of antioxidants in case group is significantly lower than that of healthy group, which shows the necessity of early diagnosis and treatment of periodontitis.

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