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Total Anti-Oxidant Capacity of Saliva in Chronic Periodontitis Patients Before and After Periodontal Treatment

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ABSTRACT

Background: Periodontal disease is among the most common inflammatory conditions which is associated with many different factors. One of the contributing factors to the pathogenesis of this condition may compromise the defensive mechanism of antioxidants. The present study evaluates the antioxidant capacity of saliva in periodontal patients before and after periodontal treatment.

Methods: In this cross sectional study, 31 patients systemically healthy non smokers with chronic periodontitis were recruited. The antioxidant capacity of saliva was measured before the initial phase of periodontal therapy and after completion of the treatment. Data were analyzed using SPSS 19 software. Paired T-Test, Independent sample T-test and ANOVA tests were used as appropriated.

Results: The mean and standard deviation antioxidant capacity of the saliva after the treatment.(0.962± 0.287µM) was significantly higher than before the treatment (0.655 ± 0.281 µM ,p<0.001). The mean difference of antioxidant capacity of the saliva before and after periodontal treatment was higher among men than among women; however, the difference was not significant (P=0.07). The mean difference of salivary antioxidant capacity was not significantly differed among different ages (P=0.772).

Conclusions: The antioxidant capacity of saliva was higher after periodontal therapy among patients with periodontal disease, however the change was not varied across the ages and gender. Therefore, the alterations in the defensive mechanism of antioxidants could be the key factors contribute to the pathogenesis of periodontal diseases.

Keywords: antioxidants; periodontitis; saliva.

INTRODUCTION

Free radicals are highly active atoms or molecules that can cause significant damage to macro molecules.[1] The human body has certain mechanisms to protect against the potential harms of free radicals (antioxidant defensive system).[2] Any alteration in the balance between the production of free radicals and the antioxidant defensive mechanisms may result in a condition called oxidative stress.[3] Some studies have documented the role of free radicals in the pathogenesis of some diseases such as periodontal diseases.

Periodontal disease is the most common inflammatory condition among human beings. Tissue injury due to free radical production has been suggested to be enhanced in individuals with periodontal disease due to a lack of adequate antioxidant defense.[4]

There is limited evidence regarding the effect of periodontal surgery on the anti-oxidant profile of saliva in patients with periodontal diseases. Therefore, this study evaluated total antioxidant capacity of saliva in patients with chronic periodontitis before and after periodontal treatment.

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METHODS

In this cross sectional study, 31 patients presenting with chronic periodontitis (CP) who referred to Zaehdan School of Dentistry Zahedan, Iran, were recruited over one year period (2012-2013). The study was approved by the Ethics Committee of Zahedan University of Medical Sciences . Patients also gave an informed consent for saliva sampling.

All patients were over the age of 30 with bleeding on probing (BOP) and pocket depth of > 5mm in at least 4-5 teeth in each quadrant. Patients meeting any of the following criteria were excluded from the study: use of any anti-inflammatory agents, vitamins (E and D), any type of synthetic antioxidants or any type of supplemental nutrients three months prior to the study, regular use of mouth rinses, pregnancy, history of smoking, systemic disease and aggressive periodontitis.

The demographic characteristics of the patients (age and sex), pocket depth, and BOP were initially recorded and stimulated saliva samples were collected in fasting conditions in base line visit. The patients subsequently underwent scaling and root planning followed by the proposed surgical procedure. The patients were called for post surgical follow-up (2 month later) and subsequent saliva samples were collected.

To stimulate the flow of saliva, the patients were asked to chew a gum for 1 minute and then spit into a 50 ml disposable tube (Pars Azmoon, Iran). The samples were combined with 50 ml of protease inhibitors and placed in centrifuge (10000 rounds/min, 4°C, 15 minutes) to separate the cellular and bacterial debris from the compound. The sample was then stored at a temperature of -20°C for subsequent analysis. After four weeks, the saliva volume was measured and the samples were sent to the biochemistry lab of Zahedan School of Medicine (Pardis University) for chemical analysis.

The saliva was evaluated in terms of the total antioxidant capacity (µM) using the Ferric Reducing Ability of Plasma (FRAP) technique. This method is based on the ability of saliva in reducing ferric ions into ferro in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ). This results in the production of a blue complex Fe-TPTZ with a maximum absorption wave length of 593 nm. The reductive ability of saliva is measured by increasing the concentration of the above-mentioned compound using spectrophotometer.

3 ml of Ferric Reducing Ability of Plasma was stored in 37°C for 10 minutes and 50 µM of the saliva sample was added to the solution. Then the mixture was stored in lab temperature for 5 minutes and its absorption was read against blank in the wavelength of 593 nm. The readings were then compared against the standard curve and the concentrations were recorded [5].

Data were analyzed SPSS19 software. Paired T-Test, Independent sample T-test and ANOVA tests were used to evaluate antioxidant capacity of saliva in periodontal patients before and after periodontal treatment.

RESULTS

The study sample consisted of 19 (61.3%) men and 12 (38.7%) women with a mean ± SD age of 44.9 ± 6.99 (range: 32-56 years).

The antioxidant capacity of saliva was significantly higher after periodontal treatment compared to before treatment (p-0.001, paired t-test,t=7.724) as in table 1

Table 1. The mean salivary total antioxidant capacity in studied patients before and after periodontal treatment (SRP & surgery).

Sampling time	number	Before surgery	After surgery
Total antioxidant capacity (µM)	31	0.281±0.655	0.287±0.962
P- Value			p-0.001

The mean antioxidant capacity of saliva before and after periodontal treatment (including SRP & periodontal surgery) with respect to age groups, gender has been shown in table2

Table 2. The mean salivary total antioxidant capacity in studied patients with respect to age groups, gender before and after periodontal treatment (SRP & surgery)

Total antioxidant capacity(µM) Subjects(gender, age groups)	Min	Max	Mean± SD
before surgery Males(n=19)	0.645	1.457	1.049±0.232
After surgery	0.187	1.206	0.686±0.291
before surgery Females(n=12)	0.165	1.441	0.824±0.321
After surgery	0.367	1.201	0.606±0.269

before surgery 31-35 years(n=4)	0.922	1.447	1.066±0.255
After surgery	0.326	1.206	0.705±0.380
before surgery36-40 years(n=6)	0.645	1.364	0.994±0.277
After surgery	0.209	1.095	0.724±0.334
before surgery41-45 years (n=6)	0.165	1.441	0.847±.0.410
After surgery	0.418	0.201	0.695±0.283
before surgery46-50 years(n=7)	0.574	1.457	0.992±0..296
After surgery	0.187	0.905	0.604±0.269
before surgery51-55 years(n=7)	0.574	1.179	0.888±0.187
After surgery	0.367	0.892	0.53±.0.194
before surgery 56-60 years(n=1)	1.345	1.345	1.345
After surgery	1.023	1.023	1.023

The mean difference in the antioxidant capacity of saliva changes before and after periodontal surgery was slightly higher in men (0.363±0.147) compared with women (0.219±0.290), table3, but according independent sample t-test the difference was not significant (P=0.07) . The one-way ANOVA result also revealed that there was no significant difference in terms of the changes in the salivary antioxidant activity before and after treatment among the different age groups (P=0.772, table3).

Table 3. The mean difference in the total antioxidant capacity of saliva changes with respect to age groups, gender before and after periodontal treatment (SRP & surgery).

Age groups, gender	30-40years (n=10)	41-50years (n=13)	50 years < (n=8)	Males(n=19)	females(n=12)
Antioxidant activity of saliva changes (µM)	0.306 ± 0.148	0.279± 0.308	0.353± 0.124	0.363± 0.147	0.219±0.290
P- Value	P=0.772			P=0.07	

DISCUSSION

Periodontal disease is the most common inflammatory condition among human beings, which may manifest as severe bone loss in patients with otherwise week oral hygiene. There appears to be factors other than periodontal pathogens contributing to the development of the disease.

It is suggested that patients with periodontitis are more susceptible to an imbalance of antioxidant , oxidative stress situation.[6,7]

Current study investigated total antioxidant capacity of saliva before and after periodontal treatment (including SRP and surgery) in patients with chronic periodontitis .This has not been previously reported . Other studies had evaluated other periodontal treatment plan including SRP, Oral hygiene instruction, etc.[6,8,9]

In this study the mean antioxidant capacity of the saliva after the treatment was significantly higher than before the treatment (p<0.001).

Novakovic, et al (2014) evaluated the influence of non-surgical periodontal treatment on salivary antioxidants . In this study all measured antioxidants were affected by treatment resulting in significant increase in Total antioxidant capacity (TAOC), ALB (albumins), UA (uric acid) and GPX (glutathione peroxides) and decrease of SOD (superoxide dismutase) in response to SRP, where no differences were observed for any of biomarkers in the oral hygiene instructions group. They concluded that non-surgical periodontal treatment affected salivary antioxidants. It seems that, these chemical biomarkers reflected periodontal status and tissue response on treatment.[8] Similarity, in current study, same to Novakovic et al study, the mean TAOC after SRP and periodontal surgery was significantly higher than before periodontal treatment /baseline visit (p-0.001)

Kim and colleagues (2010) evaluated the total anti-oxidative activity of saliva (TAS) in periodontal patients before and after root planning. They also (same to present study) revealed that TAS is lower among patients with chronic periodontitis before root planning compared to the control group.[9] Likewise, Wei and

Zhang in Nanjing Chinese (2010) showed that the total anti-oxidant activity of saliva was lower in patients with chronic periodontitis after treatment and TOS concentrations in saliva were found to be significantly higher in chronic periodontitis group compared with the control group ($p < 0.05$). After 16 weeks following non-surgical post-periodontal therapy, the concentrations of TOS in saliva decreased to the lower levels compared with the basal levels ($p < 0.05$) and showed no significant difference with the control group($p > 0.05$).[6]

Chapple et al. (2007) demonstrated that non-surgical therapy with improvements in clinical parameters can increase the antioxidant defense in chronic periodontitis patients. In their study, serum TAOC between chronic periodontitis patients and controls (p=0.57) showed no significant difference at baseline, but GCF TAOC was lower (p=0.0001) in chronic periodontitis patients than controls. They founded that Successful periodontal therapy did not alter plasma TAOC (p=0.56), but GCF TAOC increased (p=0.001) to control subject levels (p=0.47).[10] The result of Chapple et al work is similar to present study, because periodontal treatment leading to increase the antioxidant defense in chronic periodontitis patients

Likewise, in Canakci's study (2009), the findings suggested that the total antioxidant activity of saliva decreased with an increase in the level of inflammation.[11] Similarly in 2008, Guentsch and colleagues demonstrated that the total anti-oxidant activity of saliva was lower in patients with chronic periodontitis.[12] On the other hand, an earlier study in 1994 failed to reveal any significant difference in the TAS level among periodontal patients compared to healthy subjects.[13]

The present study was in line with Kim's,[9] Canaki's[11] and Guentsch's[12] findings in this respect periodontal inflammation leading to decrease the antioxidant defense in chronic periodontitis patients but

in conflict with Moore's[13] and Chapple's[14] and Wei's[6] results that failed to reveal any significant difference in the total antioxidant capacity level among periodontal patients compared to healthy subjects . The differences may be due to different methods or differences in the inclusion and exclusion criteria.

In present study performed treatment on patients was including SRP and periodontal surgery, but in other studies (Wei and Zhang(2010), Kim and colleagues (2010) patients were treated with non-surgical periodontal therapy methods such as SRP.[6,9] Novakovic et al concluded that non-surgical periodontal treatment (SRP) affected salivary antioxidants but present study showed that surgical periodontal treatment also affected salivary TAOC.[8]

In our study, although there was a decreasing pattern in the TAOC with age, we failed to reveal any significant difference in the salivary antioxidant activity among different age groups (p=0.7). But, Balkan et al (2002) observed a significantly lower antioxidant activity of the saliva among older populations.[15]

We observed no differences in total antioxidant capacity between males and females (P=0.07). Unlike in Scully, et al study, total antioxidant activity of saliva was significantly lower in women than in men (p=0.002) .The differences may be due to different in number of studied patients in two studies. They had studied 64 males and 65 females.[4]

This is the first research to demonstrate how saliva total antioxidant capacity is influenced by reductions in periodontal inflammation after successful surgical Treatment.

Chapple et al (2007) also found resolution of inflammation following successful non-surgical periodontal therapy leading to an increase in GCF TAOC.[10]

In all, our finding indicate that the antioxidant defensive may result from the periodontal inflammation.

CONCLUSIONS

The results of the current study suggest that total antioxidant capacity of saliva after SRP & surgical periodontal therapy is higher than before treatment among patients with chronic periodontal disease. Imbalance between oxidative stress and antioxidant capacity has an important role in the pathogenesis of periodontal diseases. Thus, any alterations in the defensive mechanism of antioxidants may contribute to the pathogenesis of this disease.

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