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ASSESSMENT OF GINGIVAL AND TOTAL AN TI-OXIDANT CAPACITY IN SMOKERS AND NONSMOKER'S CHRONIC PERIODONTITIS PATIENTS FOLLOWING NON-SURGICAL PERIODONTAL THERAPY

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ABSTRACT

Periodontal disease is a chronic infectious disease caused by specific bacteria and associated of inflammation of the supporting structures of teeth, there is a strong evidence for oxidative stress implication in the progression of periodontitis. Oxidative stress is a condition arising when there is a serious imbalance between the levels of free radicals in a cell and its antioxidant defenses which leads to tissue damage when antioxidant systems are unable to counteract the free radicals action efficiently Smoking also increases ROS production and is a significant source of oxidative stress. It causes depletion of systemic endogenous antioxidant capacity, resulting in increased pro-oxidant burden. Several recent studies have indicated that total antioxidant capacity (TAOC) of plasma seems to be compromised in chronic periodontitis (ChP). The aim of this study is to investigate serum and gingival crevicular fluid GCF (TAOC) in nonsmoker and nonsmoker patients with ChP to assess the effects of non-surgical periodontal therapy on the levels of (TAOC).

Methods: 20 patients are selected from the outpatient clinic in faculty of dentistry MSA university, 10 patients with severe periodontitis and were nonsmokers (group 1) while the other 10 also with severe periodontitis but were heavy smokers (group 2), both groups received non-surgical periodontal therapy, and daily oral hygiene instructions. Serum (TAOC), GCF samples and periodontal parameters (Plaque index PI, Gingival index GI, Probing depth PD and clinical attachment loss CAL are assessed at base line and one month following therapy.

Results All measures were recorded at base line and after 1 month follow up. **Serum TAOC** levels showed a significant increase in the chronic periodontitis group (group 1) following the non-surgical periodontal therapy with P value of 0.0146, also in the chronic periodontitis smoker group (group 2) the serum TAOC levels showed a highly significant increase following the non-surgical periodontal therapy with P value of 0.0025, with highly significant difference between both groups where the P value was <0.0001. **Gingival crevicular fluid TAOC** levels for group 1 showed highly significant increase following treatment with P value <0.0001, also in group 2 showed highly significant increase following treatment with P value of 0.0025, with a highly significant difference between both groups with P value <0.0001.

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For the **Gingival index**, GI, there was a highly significant difference in group 1 and group 2 at the base line and following the treatment with P value <0.0001, with a significant difference between both groups with P value of 0.0368 For the **plaque index**, PI there was a highly significance difference in group 1 with P value <0.0001 following treatment, also PI in group 2 showed a high significant difference with P value of 0.0002, on comparison of PI between both groups no significance difference was revealed P value of 0.0873 Pocket **depth PD** in group 1 and group 2 showed a significance difference following the treatment with P value <0.0001, also there was no significant difference between both groups P value of 0.0806 **Clinical attachment** level CAL in group 1 and group2 showed no significant difference following the treatment with P value <0.2916, also there was no significant difference between both groups P value of 0.2657

Conclusion In both chronic periodontitis smoker's and nonsmoker's patients, the TAOC levels in serum and gingival fluid are significantly low. Non-surgical periodontal treatment seems to reduce oxidative stress in smokers and non-smokers chronic periodontitis patients, the chronic periodontitis smokers group showed a Significant reduced TAOC in both serum and gingival fluids than the chronic periodontitis non-smokers group.

INTRODUCTION

Periodontitis is an inflammatory disease characterized by the breakdown of tooth-supporting hard and soft tissues that is initiated by specific bacteria within the plaque biofilm. periodontal disease progression relies on complex interactions between periodontopathic bacteria and the host immune system [1,2].

Free radicals and reactive oxygen species (ROS) are mandatory to many normal biologic processes. Low levels of certain free radicals and ROS lead to the growth of fibroblasts and epithelial cells in culture, whereas higher levels may result in tissue injury [3].

ROS usually cause tissue damage via multiple mechanisms, including DNA damage, lipid peroxidation (LPO), protein damage, and enzyme oxidation. The antioxidant defense systems are necessary for the removal of ROS and maintaining health. Oxidative stress appears when the dynamic redox balance between oxidants and antioxidants is intensely shifted toward oxidative potentials [3-5].

Antioxidants exist in all body fluids and tissues and protect against free radicals, antioxidants protect the cells from harmful oxidants (ROS) by removing the oxidants or repairing the damage caused by ROS in vivo.12 vitamins E, C, and A; urate; bilirubin; and substances containing Sulfhydryl groups (SH-groups) are examples of scavenging antioxidants [6, 7].

The human body incorporates a plenty of complex antioxidant systems. Therefore, the total antioxidant capacity (TAOC) assessment method has been developed to reduce the costly and time-consuming task of measuring individual antioxidant species [4].

The total antioxidant capacity (TAOC), first measured by Miller in 1993, it is a measure of the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound (Miller et al., 1993). The TAOC levels are used as biomarkers in case of chronic inflammatory conditions like Cardiovascular disease, Diabetes Mellitus, Aging, Cancers, Stress, Renal Injury etc [8].

Smoking also increases ROS production and is a significant source of oxidative stress.8 Moreover smoking causes consumption of systemic endogenous antioxidant capacity, which results in increased pro-oxidant burden [9]. Thus, protection against these species is provided by antioxidants that may be in the form of enzymes

(e.g., superoxide dismutase [SOD], catalase, and glutathione peroxidase) or low molecular weight free-radical scavengers (e.g., vitamin E, total thiol, and glutathione). The compulsory use of the body's reserves of antioxidants (AO) to detoxify the excess free radicals in smokers results in the alteration of the level of the AO [9].

MATERIALS AND METHODS

20 patients are selected from the outpatient clinic of the faculty of dentistry MSA university. All subjects were Egyptians, systemically healthy with age range from 35 to 55 years and had more than or equal to 20 teeth, with clinical attachment loss of more than to equal to 5 mm at least at one site of the affected tooth and a pocket depth more than or equal to 3 at least one site of the affected tooth, and at least one affected tooth in each quadrant.

Subjects were excluded from the study if 1) they had a course of non-steroidal anti-inflammatory drugs or antimicrobial drugs within a 3-month period before the study began; 2) were pregnant 3) had used mouthwashes or vitamin supplements within the previous 3 months.

In the group of smokers (the patient is smoking more than one pack of cigarettes per day. The patients are divided into 2 groups: Group A comprised of 10 chronic periodontitis patients. Group B comprised of 10 smoker's patients are suffering from chronic periodontitis.

Assessment of periodontal parameters:

Clinical indices were obtained at four surfaces per tooth: mesiofacial, buccal, distofacial, and lingual. PD and CAL [10] were recorded using the same probe by the same person. The most affected site for each tooth is representing the tooth i.e. sites with the higher CAL. Plaque index (PI) [11] and gingival index (GI) [12] were also assessed in the same manner. Subjects were asked to refrain from brushing within 1 hour of sampling. Clinical measures (PD, CAL, GI, PI) were recorded 1 week before plasma sampling.

Statistical analysis:

The mean values of the individual subjects were analyzed by T-test [13].

GCF sampling

Gingival Crevicular Fluid sampling to avoid contamination with saliva, only teeth of the upper jaw were selected from the most affected site. Immediately following isolation and removal of supragingival plaque, paper point (size 30) were inserted into the gingival crevice until mild resistance was felt, and kept in place for 30 seconds. The fluid volume was determined by using a Periotron (Harco, 6000), contaminated paper point with blood were excluded. Following collection of GCF the paper point placed in Eppendorf's tubes contain (300 micro liter) phosphate buffer saline and stored at -80 C till all samples are collected then, GCF was eluted from paper point by centrifugation at 3000 rpm for 15 minutes after that the paper point was removed and GCF sample kept at -40 ˚C till analysis [7, 14].

2 GCF samples are taken from each patient during the treatment procedure (one before the treatment and one following 4 weeks of the treatment.

Serum sampling

All blood samples were collected at a morning appointment after an overnight fast [15] and subjects were asked to refrain from drinking (except water) or chewing gum during that period. Compliance with abstention from these activities was checked before sampling.

Skin in the cubital fossa was sterilized with 70% alcohol and allowed to dry. A 5cc disposable syringe and 23-gauge needle was used to draw 2 ml blood from central veins by an expert nurse. Blood is centrifuged at 2500 rpm for 10 minutes and the

serum thus separated then the plasma is collected in Eppendorf and freezes at -18 till being evaluated [16]. 2 serum samples were collected from each patient one before the treatment and the other after 4 weeks of the treatment.

Assessment of TAOC:

Patient's serum and gingival crevicular fluid was assessed using the total antioxidant capacity ELISA human kit (TAC ELISA kit) which is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Human TAC. This ELISA kit for research use only, not for therapeutic or diagnostic applications!

Principle of the Assay:

TAC ELISA kit* applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-TAC antibody and an TAC-HRP conjugate. The assay sample and buffer are incubated together with TAC-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the TAC concentration since TAC from samples and TAC-HRP conjugate compete for the anti-TAC antibody binding site. Since the number of sites is limited, as more sites are occupied by TAC from the sample, fewer sites are left to bind TAC-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The TAC concentration in each sample is interpolated from this standard curve.

Statistical analysis:

The Mann-Whitney U test was used to assess the differences of plasma TAOC levels between study groups. The difference between clinical indices in group I and group II was assessed by independent t test. In addition, within-subject differences were analyzed by paired t test. Values of P <0.05 were considered as statistically significant.

RESULTS

Clinical findings

The mean PD, CAL, PI, GI of GP1and GP2 at base line and one month post treatment are presented in table (1).

TABLE (1) Mean Clinical Indices in the study Groups at Baseline, 1 Month Post-Therapy:

Parameters (sampling area)	Group	Base line SD	1 Month Post-Therapy SD	pV
PI	Gp1	2.6±0.1633	1±0.1491	<0.0001
	GP2	2.5±0.1667	1.4±0.1633	0.0002
GI	Gp1	2.2±0.1333	0.6±0.1633	<0.0001
	GP2	2.2±0.1333	1±0	<0.0001
PD mm	Gp1	4.8±0.2494	3.3±0.1528	0.0001
	GP2	5.1±0.2333	3.7±0.1528	0.0001
CAL mm	Gp1	7.9±0.2333	7.3±0.26	<0.103
	GP2	7.9±0.1795	7.7±0.26	<0.535

Laboratory findings:

The mean plasma TAOC levels for all study groups at baseline and 1 month post-treatment are presented in Table (2) The mean plasma TAOC baseline levels from patients in GP1 was 65.05 ±1.989 U/ml compared to 37.72±1.459 U/ml in GP2. This difference was highly significant (P <0.0001) Plasma TAOC levels after 1 month post-treatment increased significantly in GP1 (74.15 ±2.683 U/ml) and GP2 (45.3 ±1.591 U/ml). When comparing the results in each group before and after the treatment group 1 revealed significant increase in serum TAOC with p-value of 0.0146 and group 2 also showed significant increase with a p-value of 0.0025.

TABLE (2) Mean Plasma TAOC Levels of Study Groups in U/ml

Plasma TAOC Levels at specific time points	Group1	Group2
Base line	65.05 ±1.989	37.72±1.459
After 1 month	74.15 ±2.683	45.3 ±1.591
Group1 vs group 2 after one month	P v<0.0001	

Mean gingival fluid TAOC Levels of Study Groups in U/ml

Base line	4.12±0.267	1.97±0.1789
After 1 month	6.29±0.3121	3±0.23
Group 1 vs group2 one month	P v<0.0001	

Total antioxidant capacity in gingival fluid:

The total antioxidant capacity in gingival fluid was evaluated in both groups. In group 1, the Mean TAOC in gingival fluid before treatment was 4.12±0.267 U/ml and after treatment was 6.29±0.312 U/ml. As for group 2 the mean before treatment was 1.97±0.1789 U/ml and after the treatment was 3± 0.23. Statistical analysis between the two groups after treatment revealed high significance with a p-value <0.0001. When comparing the results in each group before and after the treatment group 1 revealed high significance in gingival fluid TAOC with p-value < 0.0001 and group 2 also showed significance with a p-value of 0.0025.

The Gingival index GI:

The mean GI in group1 before the treatment was 2.2±0.133 and 0.6±0.163 after the treatment. In group 2 the mean before treatment was 2.2±1.333 and 1±0 after the treatment. Statistical analysis between the two groups after treatment revealed significance after the treatment with p-value of 0.0368. whereas for comparison within each group revealed high significance difference following the treatment with a p-value of <0.0001 for both groups.

The Plaque index:

The mean plaque index in group 1 before the treatment was 2.6± 0.163 and 1±0.149 after the treatment.in group 2 the mean was 2.5 ±0.1667 before treatment and 1.4±0.1633 after treatment. Statistical analysis between the two groups revealed no significance after the treatment with a p-value of 0.0873 but when comparing each group before and after treatment, high significance was shown in both groups with p-value <0.0001 and 0.0002 respectively.

Clinical Attachment Level:

The mean value of CAL in group 1 before the treatment was 7.9±0.233 and 7.3±0.26 after the treatment. In group 2 the mean before treatment was 7.9±0.179 and 7.7±0.26 after the treatment. Statistical analysis between the two groups after treatment revealed no significance after the treatment with p-value of 0.2916, whereas for comparison within each group revealed no significance in attachment gain following treatment with a p-value of <0.103 and <0.535 for both groups respectively. It is noteworthy that although the CAL changes were non-significant in both groups, the CAL improvement was more in group 1 (non-smokers) when compare to group 2 (smokers).

Pocket Depth:

The mean PD in group 1 before the treatment was 4.8±0.2495 and 3.3±0.1528 after the treatment. In group 2 the mean value before treatment was 5.1±0.2333 and 3.7±0.1528 after the treatment. Statistical analysis between the two groups after treatment revealed significant difference after the treatment with p-value of 0.0806. whereas for comparison within each group revealed high significant pocket reduction before and after treatment with a p-value of <0.0001 for both groups.

DISCUSSION

The harmful effects of oxidative stress are known as oxidative damage and generally occur after exposure to a high concentration of ROS and/or a decrease in the antioxidant defense system against ROS, oxidative stress has been implicated as a major contributor in more than 100 disorders and recently, periodontitis [4].

Cigarette smoke and tar phase contain several oxidizing compounds, reactive oxygen species (ROS) and carcinogens, which damage the genome, membranes and macromolecules of cells [3-5, 17].

Few studies [18, 19] have evaluated the influence of non-surgical periodontal therapy on TAOC of GCF in chronic periodontitis patients. It is well known that determining the TAOC is one of the most convenient analysis for detecting how antioxidants counteract the oxidative stress mechanism [5].

The levels of different antioxidants in saliva, serum and GCF in health and periodontal disease have been assessed in many studies [18, 20, 21], and the effect of non-surgical therapy on antioxidant levels also have been assessed by multiple authors [4, 21, 22]. In the present study, the change in oxidative stress in periodontium following non-surgical periodontal therapy was assessed by measuring TAOC in serum and GCF.

GCF is one of the appropriate sampling methods for investigating periodontal status, because it passes through the periodontal tissues and accumulates metabolites of tissue events [20]. The TAOC assays evaluate the combined effectiveness of individual antioxidant species and may also account for the influence of antioxidant substances that are yet to be discovered or are difficult to assay. This also gives the capacity of biological systems to withstand oxidative attack [4].

The purpose of the study was to evaluate serum and GCF Total antioxidant capacity in both study groups patients before and after non-surgical periodontal therapy.

Oxidative stress resulting from exaggerated host response has been involved in the pathobiology of many human diseases involving periodontitis [24]. In health, the ROS produced from neutrophils are neutralized by antioxidant scavenging system in humans and in this process the antioxidant levels are depleted. Similarly, a reduction in oxidative stress restores the antioxidant levels representing the healthy state of tissue, in support of this concept the researchers have observed that antioxidant levels in GCF differ in periodontal disease and health [4, 21, 25,26].

The findings of the present study have shown an increase of Total antioxidant capacity TAOC in chronic periodontitis and smokers patients following non-surgical periodontal treatment.

Serum TAOC significantly increased following treatment within the nonsmoking group with p value <0.0001, and serum TAOC significantly increased following treatment with the smoker group with p value of 0.0025.

Gingival Fluid GF TAOC the total antioxidant capacity in gingival fluid was evaluated in both groups. In group 1, the Mean TAOC in gingival fluid before treatment was 4.12±0.267 U/ml and after treatment was 6.29±0.312 U/ml. As for group2 the mean before treatment was 1.97±0.1789 U/ml and after the treatment was 3± 0.23.

Statistical analysis between the two groups after treatment revealed high significance with a p-value <0.0001.

When comparing the results in each group before and after the treatment group 1 revealed high significance in gingival fluid TAOC with p-value < 0.0001 and group 2 also showed significance with a p-value of 0.0025.

This reflects that the systematically increased ROS and decreased antioxidants defenses in patients with periodontal disease, and might possibly be explained by the notion that the induced

PMNs release ROS contributing to the development of free-radical–mediated injury in periodontal tissues. Overtime, such damage may predispose to periodontal diseases. The data from the present study agree with the results of Pavlica et al. [27], who found positive relations between plasma TAOC and periodontitis in dogs. Also, our results are consistent with Brock et al. [21],15 who found that the mean total antioxidant concentrations of serum and plasma from patients with periodontal disease were lower than healthy control samples, and the results of Chapple et al. [28], who reported that GCF glutathione values and TAOC in plasma and GCF are reduced in ChP.

The number of studies investigating lipid peroxidation LPO and antioxidant AO levels in gingival tissue was small and these gingival samples were obtained during flap surgery followed by initial periodontal treatment [29-31]. The reduction of oxidative stress and increase in AO capacity after the non-surgical periodontal therapy were reported in the literature [15, 30]. In contrast D'Aiutoetal [32] demonstrated that acute increases in reactive oxygen metabolites and systemic inflammation occurred after periodontal therapy. In addition, it was suggested that periodontal therapy could not only alter the local ROS production and AO state, but also the host systemic oxidative state [32] in gingival and peripheral blood of periodontitis patients.

The results of the present study showed that Serum TAOC in smoker periodontitis group was significantly lower than the nonsmoker periodontitis group following treatment with P value <0.0001, that can be explained that tobacco smoke compromises antioxidant defenses [33. 34].

GF TAOC the total antioxidant capacity in gingival fluid was evaluated in both groups. In group 1, the Mean TAOC in gingival fluid before treatment was 4.12±0.267 U/ml and after treatment was 6.29±0.312 U/ml. As for group2 the mean before treatment was 1.97±0.1789 U/ml and after the treatment was 3± 0.23.

Statistical analysis between the two groups after treatment revealed high significance with a p-value <0.0001.

When comparing the results in each group before and after the treatment group 1 revealed high significance in gingival fluid TAOC with p-value < 0.0001 and group 2 also showed significance with a p-value of 0.0025.

The results of the present study were in accordance to with the results of Grant et al. [29], who found that non-surgical therapy restores the redox balance in patients with ChP when glutathione was measured in GCF samples before and 3 months after therapy. On the other hand, this is inconsistent with the results of Chapple et al.,16 who reported that successful periodontal therapy did not alter plasma TAOC at 3 months' post-treatment in patients with ChP. This disagreement might be caused by the different intervals used for measuring clinical parameters between the two studies [29].

The mean PD, CAL, PI, and GI of Group 1 and group 2 at baseline, and 1month post-treatment are presented in Table (1). There were significant improvements in PD, GI, PI scores at the1-month interval post-treatment in both Group1 and Group2 compared to baseline measures (P <0.0001), and these results is in accordance to results revealed by Abou Sulaiman and Shehadeh 2010 [16].

CONCLUSIONS

The present study concluded that the total antioxidant levels increased significantly following SRP from baseline to one month corresponding to improvement in clinical parameters. Similar observations were made by various authors who demonstrated improvement in total antioxidant levels in GCF following non-surgical periodontal therapy[4,13]. The improvement in TAOC levels could be explained by removal of local factors which in turn reduced the microbial load and hence reduced generation of free radicals from neutrophils.

The present study demonstrated that antioxidant profile in periodontal tissues improve following SRP and hence reducing the oxidative damage to periodontium. The present study also demonstrated that serum and GCF TOAC can be utilized as effective biomarkers for assessing periodontal status, but the therapeutic approach of administration of both local and systemic antioxidants for reducing the oxidative tissue damage in periodontium should be evaluated through long term clinical trials.

Moreover, as it was evident in the study the reduction in antioxidant capacity in smoker chronic periodontitis patients showed that they suffer from increased load of oxidative stress with impaired (antioxidant capacity) and are in need to proper and regular non-surgical periodontal therapy and additional antioxidant supplementation as an adjunctive agent to improve treatment out come

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