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A COMPARATIVE EVALUATION OF TOTAL ANTIOXIDANT LEVELS IN SALIVA OF SMOKERS AND NON-SMOKERS WITH CHRONIC PERIODONTITIS: A CLINICO BIOCHEMICAL STUDY

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ABSTRACT:

Background & Objectives: Periodontitis is an inflammatory disease of supporting tissues of the teeth. Smoking is one of the most prevalent risk factor for periodontal disease and is a major source of free radicals. The human body has an array of non enzymatic and enzymatic antioxidant defense mechanisms to remove harmful reactive oxygen species to prevent their deleterious effects. This study investigates the association between smoking and TAO levels in chronic periodontitis before and after scaling and root planing.

Materials and Methods: This case-control study enrolled 20 smokers cases and 20 non smokers controls with chronic periodontitis. Clinical parameters such as PI, GI, PD and CAL were assessed. Saliva sample was analyzed for TAO levels by spectrophotometry. Statistical analysis was done using paired‘t’ test and Pearson’s correlation analysis.

Results: The mean total antioxidant levels of smokers and non- smokers after 1 month of SRP were 45.74(SD 17.49) and 379.31(SD 140.37). A significant elevation of TAO levels were seen in both groups ($p < 0.001$).

Conclusion: Elevated levels of TAO levels were observed in both the groups. Further studies to be conducted on efficacy of antioxidant therapy along with smoking cessation programmes to determine the role of smoking on antioxidant status.

Key words: Chronic periodontitis, TAO, Smokers, Scaling and root planing.

INTRODUCTION:

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both and when left untreated resulting in tooth loss.[1]. There are various risk factors which increases the risk for periodontitis. Smoking is undoubtedly one of the most prevalent.

In general, there is substantial body of evidence to support the observation that the more a patient smokes, greater the degree of periodontal disease. Cigarette smoke is a very complex mixture of substances with over 4000 known constituents.[2] These include carbon monoxide, hydrogen cyanide, reactive oxidizing radicals, a high number of carcinogens, and the main psychoactive and addictive molecule nicotine.[3]

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Nicotine metabolites get concentrated in the periodontium and their effects include the promotion of vasoconstriction, and impairment of the functional activity of polymorphs and macrophages. Smoking has various detrimental effects on periodontal tissues. These effects include chronic reduction of blood flow, altered neutrophil function, cytokine and growth factor production, inhibition of fibroblast and growth factor attachment, decreased collagen production and gingival vascularity.[4-5].

Furthermore smoking stimulates oxidative bursts of neutrophils increases reactive oxygen species production and leads to lipid peroxidation, oxidation of protein thiols and alteration in protein carbonyls in plasma. Reactive oxygen species are toxic substances that attack and damage biologic molecles. The compulsory use of body's reserves of antioxidants to detoxify the excess of free radicals in smokers results in the alteration of the levels of antioxidants. The human body has an array of non-enzymatic and enzymatic antioxidant defense mechanisms to remove harmful reactive oxygen species to prevent their deleterious effects.[6].

Antioxidants are present in all body fluids and tissues and protect against endogenously formed free radicals usually produced by the electron transport system. Saliva is the first biological fluid that is exposed to cigarette smoke, which contains numerous toxic compositions responsible for structural and functional changes in saliva, which is very essential for oral health.[7-9] When conducting analysis of saliva for antioxidants, whole saliva is more relevant as it contains gingival crevicular fluid, immune cells and tissue metabolites.[10] Stimulated saliva has been used in the analysis of antioxidants. Since stimulation may increase the expulsion of gingival crevicular fluid from the periodontal pocket through the mastication process, this may artificially increase the concentration of antioxidants in the saliva. So, unstimulated saliva would provide a more accurate account of the saliva antioxidant composition for analysis.[11-12]

There are more than $10^{[18]}$ organic free radicals per puff in the gas phase of cigarette smoke, while the tar phase has 10^{19} free radicals per gram. [13]

The obligatory use of body's reserve of antioxidants to detoxify the excess of free radicals in smokers results in alteration in the level of different antioxidants. The antioxidant disturbance in smokers may be further enhanced by their lower intake of both supplemental and dietary antioxidants. [14] Phase I therapy is the first step in the chronologic sequence of procedures that constitute periodontal treatment. The objective of Phase I therapy is to alter or eliminate the microbial etiology and contributing factors for gingival and periodontal diseases.

Pryor *et al* and Palmer *et al* found that the reduction in total antioxidant concentration in smokers compared with the non-smokers with periodontitis is due to the local impact of the additional reactive oxygen species (ROS) created through the chronic use of tobacco. [15-16] It may therefore be that smokers may have a compensatory protective mechanism to deal with the additional local oxidative stress, whereby antioxidant systems are up-regulated. The removal of the inflammation and associated oxidative stress, results in a stronger recovery of total antioxidant capacity locally in smokers, despite apparently greater residual inflammation post-therapy. Thus the aim of this study was to estimate the change in levels of total antioxidants in saliva of smokers and non-smokers with chronic periodontitis after performing scaling and root planing (SRP).

MATERIALS AND METHODS:

Source of data

The subjects for the study were selected from patients visiting the Department of Periodontics and Implantology at Vydehi Institute of Dental Sciences and Research Centre, Whitefield, Bangalore for routine dental examination. Written informed consent was taken from the subjects who were recruited for the study. It was a randomized controlled study. A total of forty systemically healthy individuals between 25-40 years of age, were included in the study. The subjects that were selected for the study were diagnosed with moderate to severe chronic generalized periodontitis. The subjects were divided into two groups of twenty subjects each:-

Group I (smokers)– Moderate to severe chronic periodontitis

Group II (non-smokers)– Moderate to severe chronic periodontitis

Athorough clinical periodontal examination was carried out and the parameters selected for the study were carefully recorded. All the recording was done by a single examiner.

The clinical parameters recorded were:

1. Plaque index {Sillness and Loe (1964)}

2. Gingival index {Loe and Sillness (1963)}

3. Full mouth probing pocket depth

4. Full mouth clinical attachment level.

Inclusion criteria [17-18]

Patients with age range 25-40 years.

Smokers who smoked atleast > 10 cigarettes/day for more than / a minimum of 5 years.

Presence of more than twenty functional teeth should be present at the time of study.

Moderate to severe chronic periodontitis (>3mm CAL).

Exclusion criteria

Patients with any known systemic disease and conditions.

Patients with history of any periodontal therapy within the past 6 months.

Patients with any history of drug intake in past 3 months.

Pregnant and lactating mothers.

Saliva sample collection

Unstimulated whole saliva samples were collected prior to the periodontal therapy. Patient was seated on the dental chair and saliva samples was collected over a 5 min period with instructions to allow saliva to pool in the floor of the mouth and collection was done into poly-propylene tubes. Saliva samples collected was centrifuged at 4000g for 10min at 40°C.Supernatant fraction was then stored at -800 C[18]

The total antioxidant levels of saliva samples was determined in whole saliva by ferric reducing ability of plasma assay (FRAP) assay according to the method of Benzie et al.[19]

Statistical analysis

The statistical significance of differences in salivary antioxidant levels between smokers and non-smokers was estimated by t-test and Pearson correlation . In this study 'p' value less than 0.05 were accepted as significant.

RESULTS:

The mean levels of PI, GI, PD and CAL were found to be significantly less in both smokers and non-smokers after SRP when compared to the baseline values. (Table A.1,2,3,4,5)

There was a statistically significant inverse correlation between total antioxidant levels in saliva and age in both the groups, indicating that as the age increases, total antioxidant levels would decrease (Table A.6,Graph B1,B2). Also there was a significant negative correlation between total antioxidant levels and number of cigarette smoked indicating that when the number of cigarettes smoked increased, the total antioxidant levels in saliva would decrease (Table A.7,Graph B3).

DISCUSSION:

In the present study total antioxidant levels in saliva were assessed in 40 patients, comprising of 20 smokers and 20 non-smokers with chronic periodontitis before and after scaling and root planning(SRP). Unstimulated whole saliva was obtained at baseline and after SRP in both the groups.

In the present study on comparison with smokers and non-smokers the mean PI,GI,PD and CAL at baseline and 1month after SRP the results of both the groups showed a statistical significance with p-value of <0.001, where the PI value were increased in smoker group when compared to the non-smoker group at baseline [20-23]. The study done by Calsina et al found smokers exhibited greater probing depth

and clinical attachment loss when compare to non-smokers with chronic periodontitis.[24]

In the present study the mean total antioxidant levels at baseline and after 1month was compared between smokers and non-smokers showed a statistical significance with p-value of <0.001.[25] The above results were in contrast to the study done by B.Rai et al where he determined the association between total glutathione levels, in saliva in smokers and non-smokers with periodontitis.[26]

In our study both the groups showed a significant negative correlation between TAO levels and age(r= -0.97). Studies have shown that TAO levels is gradually reduced with aging in smokers as well as in non-smokers which could be due to the release of free radicals as the age progresses. This is in agreement with the study done by Baydaa et al.[27]

When the TAO levels and number of cigarettes were correlated in the present study, an inverse correlation (r=-0.87) between the two parameters was seen. That is when the number of cigarettes smoked increased, the TAO levels in saliva decreased. This was in accordance with the study done by Bassam et al.[28] Tobacco induces alterations in microbial and host factors which inturn contribute to deleterious effects on the periodontium.[29-30] Hence dental health professionals should advice patients of tobaccos negative health effects as well as the benefits of quitting tobacco use and tobacco counselling should be a part of treatment plan for every patient who has the habit of using tobacco and its products.

Thus the comparison of TAO levels in saliva of periodontitis with or without smoking has imbalance in TAO levels.

CONCLUSION:

Periodontitis is a destructive chronic inflammatory disease which results from the loss of delicate balance between microbial virulence factors and host response. Smoking has long been recognized as a risk factor for periodontal disease and a great deal of research into the detrimental effects of tobacco smoking have concluded that it has widespread systemic effects, many of which may provide mechanisms that increase the individual patients susceptibility to periodontal disease and affect their response to treatment, by stimulating destructive/inflammatory responses and impairing protective/reparative responses.

The present study aims to estimate the total antioxidant levels in saliva of smokers and non-smokers with chronic moderate to severe periodontitis and also to analyze the changes in levels of TAO in saliva before and after SRP.

Following conclusions may be drawn from this study:

The levels of total antioxidants in both smokers and non-smokers showed statistical significant increase in saliva

after scaling and root planing. It has been attributed to the resolution of inflammation and thereby reducing free radical release ultimately improving the total antioxidant status by performing scaling and root planing.

Significant negative correlation was found between the number of cigarettes smoked and total antioxidant levels. This may be related to the adverse effect of smoking that every time a cigarette is smoked, there is an increase in the oxidative stress.

Significant negative correlation was also found between age and total antioxidant levels in both smokers and non-smokers. It may be due to increased free radical release as the age increases, which in turn bring about a reduction in total antioxidant levels.

Since the duration of the present study was for only a period of one month, motivation for cessation of smoking was not possible, so further studies with larger sample size should be considered which would have minimized the errors in assessing the imbalance in total antioxidant levels between Group I and Group II patients.

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TABLES:

Plaque index	Baseline Mean ±SD	After SRP (1 month) Mean ±SD	Difference From baseline	p- value
GROUP I	1.89 ±0.29	1.01±0.06	0.88	<0.001
GROUP II	1.90 ±0.25	1.04±0.20	0.86	<0.001

Table A.1: Mean, standard deviation and p-value for plaque index in Group I and Group II.

Gingival index	**Baseline Mean ±SD**	**After SRP (1 month) Mean ±SD**	**Difference from baseline**	**p- value**
GROUP I	2.00±0.5	1.02±.05	0.98	<0.001
GROUP II	1.92 ±0.36	1.06 ±0.19	0.86	<0.001

Table A.2: Mean, standard deviation and p-value for gingival index in Group I and Group II

Pocket depth	**Baseline Mean ±SD**	**After SRP(1 month) Mean ±SD**	**Difference from baseline**	**p- value**
GROUP I	3.96 ±0.9	2.62±0.73	1.34	<0.001
GROUP II	4.04 ±1.12	2.34± 0.83	1.7	<0.001

TableA.3: Mean, standard deviation and p-value for pocket depth in Group I and Group II.

Total antioxidant levels	Baseline Mean ±SD	After SRP (1 month) Mean ± SD	Difference from baseline	p-value
GROUP I	34.63±13.922	45.74±17.49	11.11	0.007
GROUP II	285.29 ±56.11	379.31± 140.37	94.02	0.001

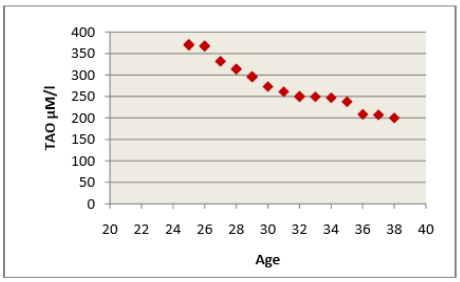
Table A.4: Mean, standard deviation and p-value for clinical attachment levels in Group I and Group II.

Clinical Attachment levels	Baseline Mean ±SD	After SRP(1 month) Mean ±SD	Difference from baseline	p-value
GROUP I	3.15±0.76	2.62±0.73	1.34	<0.001
GROUP II	3.86 ±1.17	2.27± 1.04	0.59	<0.001

Table A.5: Mean, standard deviation and p-value for total antioxidant levels in Group I and Group II

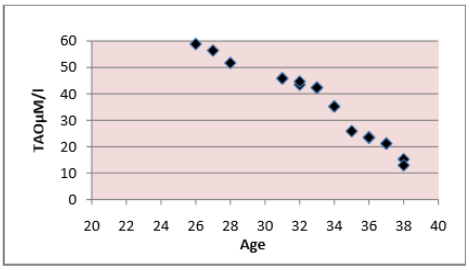
	TAO levels Mean ±SD	Age Mean ±SD	Pearson correlation r
Group I	34.63±13.92	3.65±3.72	-0.97221
Group II	285.3±56.1	30.8±3.49	-0.97

Table A.6: Pearson correlation between TAO levels and Age in Group I and Group II.



Group I (smokers)

GRAPH B.1

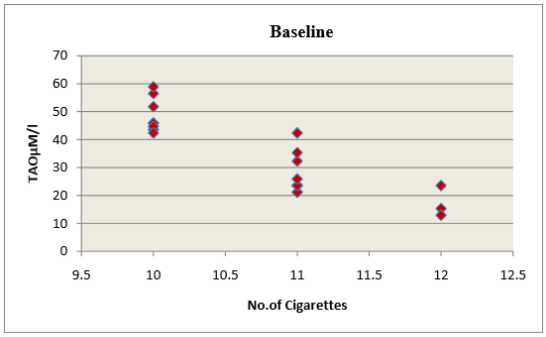


Group II (non-smokers)

GRAPH B.2

	FRAP Mean ±SD	No. of cigarettes Mean ±SD	Pearson correlation r
Group I	34.63±13.92	10.75±0.72	-0.87

Table A.7: Pearson Correlation between TAO levels and number of cigarettes smoked in Group I.



GRAPH B.3: Correlation between TAO levels and no. of cigarettes smoked in Group I.