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ORIGINAL RESEARCH

Assessment of Lipid Peroxidation Levels and Total Antioxidant Status in Chronic and Aggressive Periodontitis Patients: An *in vivo* Study

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ABSTRACT

Introduction: Periodontitis is a common problem affecting a significant population of the world. For the assessment of oxidative stress of an individual, total oxidation status (TOS) and total antioxidant capacity (TAOC) are the significant biomarkers. Hence, we planned the present study to assess malondialdehyde (MDA), TOS, TAOC levels, and oxidative stress index (OSI) in generalized aggressive periodontitis (GP) and chronic periodontitis (CP) patients.

Materials and methods: The present study included assessment of 40 CP patients, 40 GP patients, and 40 healthy controls. Clinical assessment of all the subjects was done by measuring the probing depth (PD), clinical attachment (CL), gingival index (GI), gingival bleeding index (GBI), and plaque index (PI). Salivary and serum samples were taken and assessed by standard procedures as described previously in the literature. All the values were assessed and compared.

Results: Significant results were obtained while comparing all the periodontal parameters in between various study groups. Mean serum MDA levels in the CP, GP, and control group were found to be 0.68, 0.65, and 0.61 μM respectively. Statistically nonsignificant results were obtained while comparing the serum MDA levels in between the three study groups. Significant results were obtained while comparing the mean serum and salivary TOS values, TAOC values, and OSI in between various study groups.

Conclusion: In periodontitis patients, oxidative stress was significantly higher in comparison with healthy subjects.

Clinical significance: Oxidative parameters do play a significant role in the pathologic profile of periodontitis.

Keywords: Chronic periodontitis, Generalized aggressive periodontitis, Oxidative stress.

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INTRODUCTION

In recent past years, saliva has become a routine component of diagnostic techniques in the field of clinical research and diagnostic pathology. Its noninvasive nature, easy method of collection, and ease of availability make it more advantageous over other diagnostic fluids.[1,2] Before establishing it in routine clinical practice, its specificity and sensitivity have to be established. The two most common diseases of the oral cavity are periodontitis and dental caries.[3] Biomarkers are a group of parameters that allow early detection and help in establishment of diagnosis and prognosis of a disease. For the diagnosis of oxidative stress, optimal biomarkers should be both sensitive and specific.[4,5]

Lipid peroxidation (LPO) is most frequently studied in terms of MDA levels. Total oxidation status and TAOC are the significant biomarkers for the assessment of oxidative stress of an individual. Another important oxidative parameter for assessing the oxidative stress includes OSI. In the literature, the difference and similarity in the oxidative profile of GP and CP are not clearly established.[6-8] Hence, we planned the present study to assess the oxidative stress biomarkers in patients with CP and GP.

MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology of the Dental Institute and included assessment of 120 subjects. Ethical approval was taken from Institutional Ethical Committee and written consent was obtained after explaining in detail the entire research protocol. Of 120 subjects included in the present study, 40 subjects were of CP, 40 subjects were of GP, and the remaining 40 subjects were taken as healthy controls. All the groups consisted of equal number of males and females. All the patients included in the present study reported to the college outpatient department for check-up of routine periodontal problems. Following the criteria given by the American Academy of Periodontology, complete clinical and radiographic evaluation of all the subjects was done for the diagnosis of CP and GP.[9,10] The following clinical and periodontal criteria were used for establishing diagnosis of both the study groups.

Chronic Periodontitis Group

Patients having more than or equal to 30% of periodontal bone loss, with teeth having level of CL of ≥5 mm, along with periodontal PD of ≥5 mm at single or multiple sites on teeth at more than one site of all tooth quadrants.

Generalized Aggressive Periodontitis Group

Patients within the age group of 18 to 40 years, having ≥20, having CL of ≥6 mm and PD of ≥6 mm at one or multiple sites of ≥12 teeth.

Control Group

Control group consisted of subjects with healthy periodontal status as characterized by no history of periodontal diseases, with a PD of ≤3 mm, CL of ≤1, good oral hygiene, and absence of gingival inflammation.

Exclusion criteria for the present study were as follows:

- Patients <18 years of age,
- Patients with a history of any systemic illness,
- Patients with any known drug allergy,
- Patients who had taken any form of antibiotic therapy, or anti-inflammatory therapy in the past 3 months,
- Patients with smoking habit.

Clinical assessment of all the subjects was done by measuring the PD, CL, GI, GBI, and PI based on criteria previously described in the literature. All the examination procedures were performed by a single examiner.[11-13]

Collection of Salivary and Serum Samples

After performing the clinical measurements, 48 hours later, the salivary samples were collected in the morning after overnight fasting. In the morning, on the day of collection of samples, the patients were instructed not to eat or drink anything until the sample was taken. In the present study, unstimulated salivary samples were used. Collection of salivary sample was done over a 5 minute procedure, and the patients were instructed to let the pooling of saliva in the floor of the mouth followed by draining to a collection tube, whenever necessary. Patients were prohibited for swallowing of the saliva for calculation of salivary flow rates (SFRs). By dividing the quantity of saliva obtained, with time, we obtained SFR.[14] Centrifugation of salivary sample was done at 4,000*g* for 10 minutes at 4°C. This was followed by storage of the samples in the storage vials.[15] For collection of venous blood, plain tubes were used, which were maintained at 4°C for half an hour followed by centrifugation. Cryogenic vials were used for storing the serum samples. Estimation of salivary and serum MDA levels was done by MDA (LPO) assay, previously described by Young and Trimble.[16] Method described by Erel[17] was used for the evaluation of serum and salivary TOS levels. For evaluation of TAOC salivary and serum levels, enzyme-linked immunosorbent assay (ELISA) was used as per manufacturer's instructions. For the calculation of OSI, we used the percentage ratio of TOS to TAOC.[17]

All the results were compiled and analyzed in Statistical Package for the Social Sciences software. Chi-squared test, Student's t-test, and Mann–Whitney U-test were used for the assessment of level of significance; $p < 0.05$ was taken as significant.

RESULTS

In the present study, we evaluated a total of 120 subjects and divided them broadly into three study groups: CP group, GP group, and the control group. The mean PD in CP, GP, and control group was found to be 3.75, 4.62, and 1.02 mm respectively. Significant results were obtained while comparing all the periodontal parameters in between various study groups ($p < 0.05$; Table 1). However, we did not observe any significant difference in the SFR of the subjects of various study groups ($p > 0.05$).

Mean serum MDA levels in the CP, GP, and control group were found to be 0.68, 0.65, and 0.61 µM respectively (Table 2). Statistically nonsignificant results were obtained while comparing the serum MDA levels in between the three study groups ($p > 0.05$). Comparative evaluation of serum and salivary TOS levels in between various study groups is highlighted in Table 3. Mean value of serum TOS in the CP, GP, and the control group was found to be 17.5, 22.1, and 14.12 µM H_2O_2 equivalent respectively. Significant results were obtained while comparing the mean serum and salivary TOS values in

Table 1: Comparative evaluation of various clinical periodontal parameters among subjects of all the study groups

Parameter	*Groups*	*Median*	*Chi-square value*	*p-value*
PD (mm)	CP	3.75#^	84.63	0.02*
	GP	4.62#		
	Control	1.02		
CL (mm)	CP	4.19#^	86.14	0.03*
	GP	5.20#		
	Control	0.83		
GI	CP	1.53#^	84.22	0.01*
	GP	2.31#		
	Control	0		
GBI	CP	1.52#^	83.12	0.02*
	GP	2.42#		
	Control	0		
PI	CP	1.02#^	79.25	0.01*
	GP	2.08#		
	Control	0		
SFR	CP	0.38	5.25	0.25
	GP	0.40		
	Control	0.37		

*Significant; #significant difference in comparison with the control group; ^significant difference in comparison with the GP group

between various study groups ($p < 0.05$). Comparative evaluation of serum and salivary TAOC levels in between various study groups is shown in Table 4. Mean serum TAOC values in the CP, GP, and control group subjects were found to be 1.10, 1.04, and 1.50 mM Trolox equivalent respectively. In the CP, GP, and the control group, the mean salivary TAOC values were found to be 0.60, 0.52, and 0.78 mM Trolox equivalent respectively. We observed significant difference while comparing the mean serum and salivary TAOC values in between various study groups ($p < 0.05$). Significant results were obtained while comparing the serum and salivary OSI in between various study groups (Table 5).

DISCUSSION

One of the common health problems affecting approximately 10% of the global population is periodontitis. There exists considerable variation in its prevalence due to variation in both demographic and personal details of the area population. Reactive oxygen species have arisen as significant signaling molecules in different cellular processes. These molecules are instigated from molecular oxygen and principally yield cellular damage, if not neutralized by antioxidant substances. Their formation is a crucial constituent of the host response to a number of noxious stimuli, including bacteria. Oxidative damage can be best predicted by LPO biomarkers.[7,8] Hence, we planned the present study to assess the oxidative stress biomarkers in patients with CP and GP.

In the present study, we observed significant differences while comparing the periodontal parameters in between the three study groups ($p < 0.05$) (Table 1). Attachment loss was significantly more in patients of GP study group. Our results were in correlation with the results obtained by Baltacıoğlu et al,[18] who also reported similar findings in their study. In a prospective study, Akalin et al[14] investigated the MDA levels and TOS values in saliva, serum, and gingival crevicular

Table 2: Comparative evaluation of serum and salivary MDA levels in between various study groups

Parameter	*Groups*	*Mean*	*Chi-square value*	*p-value*
Serum MDA (μM)	CP	0.68	0.702	0.336
	GP	0.65		
	Control	0.61		
Salivary MDA (μM)	CP	0.16	35.02	0.04*
	GP	0.16		
	Control	0.07		

*Significant

Table 3: Comparative evaluation of serum and salivary TOS levels in between various study groups

Parameter	*Groups*	*Mean value*	*Chi-square value*	*p-value*
Serum TOS (μM H_2O_2 equivalent)	CP	17.5	60.25	0.01*
	GP	22.1		
	Control	14.12		
Salivary TOS (μM H_2O_2 equivalent)	CP	6.56	81.25	0.02*
	GP	7.75		
	Control	4.30		

*Significant

Table 4: Comparative evaluation of serum and salivary TAOC levels in between various study groups

Parameter	*Groups*	*Mean value*	*Chi-square value*	*p-value*
Serum TAOC (mM Trolox equivalent)	CP	1.10	71.25	0.03*
	GP	1.04		
	Control	1.50		
Salivary TAOC (mM Trolox equivalent)	CP	0.60	26.35	0.04*
	GP	0.52		
	Control	0.78		

*Significant

Table 5: Comparative evaluation of serum and salivary OSI levels in between various study groups

Parameter	*Groups*	*Mean value*	*Chi-square value*	*p-value*
Serum OSI	CP	1.6	44.25	0.02*
	GP	2.20		
	Control	0.92		
Salivary OSI	CP	1.7	39.52	0.01*
	GP	0.62		
	Control	1.30		

*Significant

fluid (GCF) of the CP patients. They evaluated a total of 36 CP patients and 28 control subjects and did their clinical assessment. Sampling was done in all these subjects and evaluation of MDA and TOS levels was done by liquid chromatography. While assessing the mean serum MDA levels in between the study group and the control group, they did not observe any significant difference. However, significant difference was observed while comparing the salivary and GCF MDA level in between the study group and control group. However, compared with the control group, the authors observed significantly higher values of serum and salivary TOS in the CP group. From the above results, the authors concluded that a key role is played by LPO and TOS in the pathology of periodontitis. Furthermore, significant results were obtained while comparing the mean serum TOS, TAOC, and OSI levels in between the study group and the control group (Tables 4 and 5). A significant product of LPO is MDA. In a study conducted by Baltacıoğlu et al,[18] levels of MDA, TOS, TAOC, and OSI were evaluated in the serum and saliva of periodontitis patients. They assessed a total of 98 patients and divided them into three study groups; CP group with 33 patients, GP group with 35 patients, and healthy control group with 30 patients. Liquid chromatography method was used for the estimation of MDA, TOS, and TAOC levels, while they used calorimetric method for clinical assessment of the samples. For the calculation of the OSI values, they used the formulae "TOS/TOAC × 100" for the estimation of mean OSI values. Significantly higher levels of salivary MDA and serum and salivary TOS and OSI were observed in the periodontitis group in comparison with the control group. In comparison with the control group, significantly lower levels of serum and salivary TAOC levels were observed in the periodontitis group. However, they did not observe any significant difference in the mean values of serum MDA levels. Overall, the oxidative stress was comparatively higher in the GP group in comparison with the CP group. On correlating the periodontal parameters and the oxidative parameters, significant positive and negative correlations were observed. From the results, the authors concluded that in the pathology of periodontitis, a significant role is played by elevated TOS and decreased TAOC.[18]

On comparing the mean salivary MDA, TOS, TAOC, and OSI levels in between the three study groups, significant results were obtained (Tables 2 and 3). Similar results were reported in the past literature.[18] Baltacıoğlu et al[19] investigated the correlation between the TOS and receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) levels in CP and GP patients. They assessed 30 GP patients, 30 CP patients, and 28 healthy controls. Automatic colorimetric method and ELISA methods were used for the estimation of serum and GCF TOS, RANKL, and OPG levels. They observed that in comparison with the control group, the patients with periodontitis had higher value of mean serum and GCF levels of the above-mentioned parameters. From the results, they concluded that severity of periodontitis is closely related to the oxidative stress. Superoxide dismutase concentration, TOS, and MDA levels in periodontal patients were examined by Wei et al.[20] They also examined the impact of periodontal therapy on the index levels in CP patients. After analyzing the CP patients and controls, they observed that in the periodontal region, LPO levels were significantly higher.

In another study conducted by D'Aiuto et al,[21] 145 periodontitis patients and 56 healthy controls were evaluated. Assessment of diacron reactive oxygen metabolites (D-ROM), antioxidant potential, C-reactive protein, interleukin-6, and lipid profiles was done in all the patients at various time intervals. Higher D-ROM levels were observed in subjects with severe periodontitis. Their results depicted a positive correlation between periodontitis and oxidative stress.

CONCLUSION

Significantly higher amount of oxidative stress is found in periodontitis patients in comparison with healthy subjects. The GP patients are subjected to higher oxidative stress than CP patients. Therefore, these oxidative parameters do play a significant role in the pathologic profile of periodontitis. However, future studies are required with higher sample size and more number of parameters for better exploration of this field of periodontal medicine.

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