



RESEARCH ARTICLE

SHORT TERM EFFECTS OF NON-SURGICAL PERIODONTAL TREATMENT ON PLASMA LEVEL OF REACTIVE OXYGEN METABOLITES IN PATIENTS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Introduction: Chronic Periodontitis, an inflammatory disease caused by oral bacteria stimulates the host cells, neutrophils which release Reactive Oxygen Species (ROS) as a part of immune response. Excess ROS is one of the pathological features in the periodontal lesion. Recently, Reactive Oxygen Metabolites (ROM) were recognized as a useful measure for blood ROS. To reduce inflammation, periodontal therapy is required. Therefore nonsurgical periodontal therapy- Scaling and Root Planing (SRP) may be effective in decreasing circulating ROS.

Aims and Objectives: The purpose of this study is to estimate the plasma ROM level in healthy and diseased periodontium and the effects of nonsurgical periodontal therapy- Scaling and Root planing on plasma ROM level in Chronic Periodontitis.

Materials and Methods: The study population consisted of 20 subjects belonging to both sexes were randomly selected. Subjects were divided in two groups, Healthy periodontium (GROUP I) and Chronic Periodontitis (GROUP II). Plaque index (PI), Sulcular bleeding index (SBI), Probing Pocket Depth (PPD), Clinical attachment level (CAL) were recorded at baseline, 1st and 2nd month. Blood was collected to assess the plasma ROM level at baseline in both groups. After SRP, in group II subject's blood was collected at 1st and 2nd month to assess plasma ROM level.

Results: The mean plasma ROM level in group I subjects was 162.6 CARRU and in group II subjects 321 was CARRU at baseline. Comparison of clinical parameters and ROM between test group and control group at baseline was significant (P<0.001). In test group, correlation between ROM level and plaque index was significant (P= 0.026). The other clinical parameters and ROM level at baseline, 1st month and 2nd month were non-significant.

Conclusion: The present study suggests that non-surgical periodontal therapy -Scaling and Root planing was effective in improving clinical parameters and reducing plasma ROM level in Chronic Periodontitis subjects.

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INTRODUCTION

Periodontitis is an inflammatory lesion mediated by complex host parasite interactions, that leads to the loss of connective tissue attachment to root surface cementum and adjacent alveolar bone.ⁱ The inflammatory and immune responses to the bacteria and also viruses that colonize the periodontal and associated tissues involve the systemic circulation and ultimately the peripheral systems of the body. This creates a complex bi-directional series of host microbial interactions involving cellular and humoral factors and networks of

cytokines, chemokines and growth factors.ⁱⁱ Neutrophils play a pivotal role in host defense and are the first line of defense against these infectious periodontal diseases. Neutrophils have several selective mechanisms for controlling bacterial invasion, including both intracellular and extracellular oxidative and non-oxidative killing mechanisms.ⁱⁱⁱ The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS).^{iv} In the various etiopathogenesis of the periodontal pathology, besides the inflammatory, immune, microbial, local or general vascular processes and environmental factors are also involved reactive oxygen species and nitrogen species either as trigger agents or more frequently aggravating the primary lesions.^v Free radicals are a family of highly reactive and diverse species capable of

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extracting electrons and thereby oxidizing a variety of biomolecules vital to cell and tissue function, which not only includes oxygen free radicals, but also nitrogen and chlorine species. Therefore the body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants as soon as they form, or to repair the damage caused by ROS in vivo^{vi}. In normal physiology, there is dynamic equilibrium between ROS activity and antioxidant defense capacity and when that equilibrium shifts in favour of ROS, either by reduction in antioxidant defences or an increase in ROS activity production or activity, oxidative stress results. Reactive oxygen metabolites (ROM) are recognized as a useful measure of blood ROS^{vii}. A variety of surgical and nonsurgical treatment modalities are available for the management of inflammatory periodontal diseases. Subgingival scaling and root planing (SRP) is the most important procedure and clinical efficacy has been demonstrated in numerous clinical studies^{viii}. This study was carried out to estimate the level of plasma reactive oxygen metabolites in healthy and diseased periodontium and to assess the effect SRP on plasma ROM levels in chronic periodontitis.

MATERIALS AND METHODS

The study population consisted of twenty subjects belonging to both sexes and all the subjects were randomly selected from the outpatient clinic of the Department of Periodontics, Thaimoogambigai Dental college, Chennai. The study protocol was approved by the ethical committee of Dr. MGR University. Subjects were divided into two groups of ten subjects each as Group I – healthy periodontium and Group II – Chronic periodontitis. The selection criteria for healthy periodontium included good oral hygiene, no visual signs of gingival inflammation, probing pocket depth (PPD) of < 3mm at all sites of all teeth and no evidence of attachment loss. The selection criteria of chronic periodontitis included at least four teeth with one or more sites exhibiting probing depth > 4mm and clinical attachment loss > 4mm. The exclusion criteria for both the groups were pregnancy, previous or current smokers, use of antioxidant supplements, anti-inflammatory medications or antibiotics taken within previous three months. A null hypothesis of no difference between control group and test group was set at the beginning of the study. Clinical periodontal parameters assessed for both the groups were plaque index, (Silness & Loe), Modified Sulcular Index (Mombelli *et al*), Probing pocket depth and Clinical attachment level at baseline. Blood was also collected for both the groups for the analysis of plasma ROM. Scaling and root planing was performed for Group-II. The treatment consisted of an average of four, forty five minute sessions over 3 weeks. The SRP was performed with gracey curettes. Clinical parameters were also assessed at first and second month after therapy. Plasma ROM levels were also estimated at first and second month after SRP.

Measurement of Plasma ROM level

This was in accordance with the study done by Tamaki *et al* in 2009^{ix}. Two ml of blood was drawn from the lateral aspect of the cubital vein with needle and syringe. The blood was transferred to a test tube and then centrifuged at 3000 x g for five minutes. Add 50µl of plasma sample to 50µl of horse radish peroxidase. Mix well and then add 50µl of tetra methyl benzene substrate. Then finally add 100µl stop solution. Read it in spectrophotometer at 450nm against 620nm immediately. The plasma ROM level was measured by carratelli unit (CARR

U) and it was established that 1 CARR U corresponds to 0.08mg/dl hydrogen peroxide.

RESULTS

The data was collected for various parameters such as plaque index, sulcular bleeding index, probing depth, clinical attachment loss. These were recorded on a previously prepared proforma for the study. The data was analysed statistically to find the mean, standard deviation, and test of significance of mean values, for the various parameters between the groups. The independent sample t-test was used to compare mean values between test and control groups and to calculate the P-value and the Repeated measures ANOVA was used to compare the ROM levels and clinical parameters in the test group. Results in the present study showed ROM levels of control group at baseline is (162.6 ± 10.5) Caratelli unit and ROM levels of test group at baseline (321 ± 47.54) and at first month (209.7 ± 29.48) and at second month (139.3 ± 12.7) depicted in Table 1 and Table 2. Comparison of ROM levels in the test groups at different time points using repeated measures ANOVA is highly significant (P< 0.001) and is shown in Table 3. All the mean ROM levels between test and control group at baseline using independent sample T-test showed highly significant differences (P<0.001) depicted in Table 3. The mean Plaque index levels in test groups at baseline is 2.453 ± 0.342, 1st month is 1.340 ± 0.250, 2nd month 0.590 ± 0.255 is depicted in Table 2. On comparing of mean Plaque index at different time points showed significant P-value (P< .001). The mean SBI levels in test groups at baseline is 2.353 ± 0.302, 1st month is 1.473 ± 0.149, 2nd month 0.550 ± 0.165.

Table 1. Comparison of clinical parameters and Reactive Oxygen Metabolites between test and control groups at Base line

Clinical parameters	Group	N	Mean	Std. Dev	t-Value	P-Value
Plaque Index	Test	10	2.453	0.343	16.121	<0.001
	Control	10	0.510	0.166		
SBI	Test	10	2.353	0.302	17.338	<0.001
	Control	10	0.620	0.092		
Probing Depth	Test	10	4.067	0.306	14.148	<0.001
	Control	10	2.350	0.232		
CAL	Test	10	4.257	0.124	39.089	<0.001
	Control	10	2.210	0.110		
ROM	Test	10	321.0	47.5	10.287	<0.001
	Control	10	162.6	10.5		

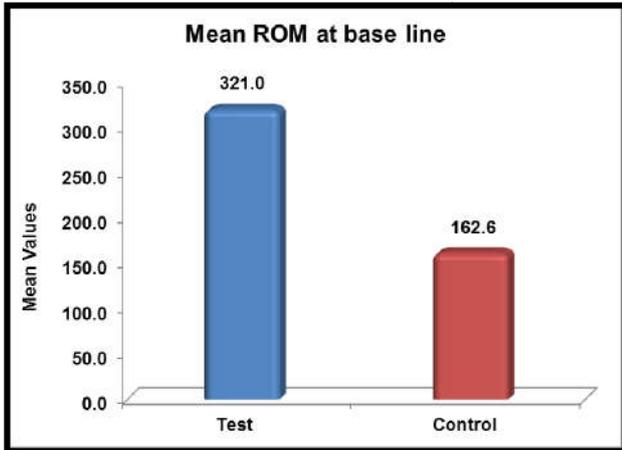
Table 2. Clinical Parameters in the test group at different time intervals

Clinical Parameter	Time Period	N	Mean	Std dev	p-value
Reactive Oxygen Metabolites	Baseline	10	321.0	47.54	<0.001*
	1 st month	10	209.70	29.48	
	2 nd month	10	139.3	12.7	
Plaque index	Baseline	10	2.453	0.342	<0.001*
	1 st month	10	1.340	0.250	
	2 nd month	10	0.590	0.255	
Sulcular Bleeding Index	Baseline	10	2.353	0.302	<0.001*
	1 st month	10	1.473	0.149	
	2 nd month	10	0.550	0.165	
Probing pocket depth	Baseline	10	4.067	0.306	<0.001*
	1 st month	10	3.200	0.200	
	2 nd month	10	2.519	0.215	
Clinical Attachment Level	Baseline	10	4.257	0.124	<0.001*
	1 st month	10	3.172	0.295	
	2 nd month	10	2.570	0.273	

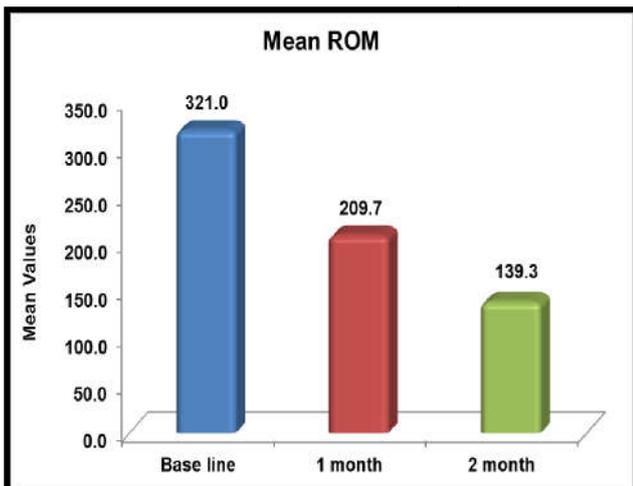
*- significant (p<0.05)

Table 3. Correlations between Reactive Oxygen Metabolites level and clinical parameters in test group

Parameters	Baseline		1 st month		2 nd month	
	p-value	R	p-value	R	p-value	R
PI	0.026	0.694	0.258	-0.396	0.135	0.507
SBI	0.573	-0.203	0.161	-0.479	0.810	0.087
CAL	0.857	0.066	0.051	0.630	0.410	0.294
PD	0.960	0.018	0.816	0.085	0.253	-0.400



Graph 1. Mean Reactive Oxygen Metabolites at baseline in test and control



Graph 2. Mean Plasma Reactive Oxygen Metabolites in test group



Figure 1. Heparinized blood centrifuged

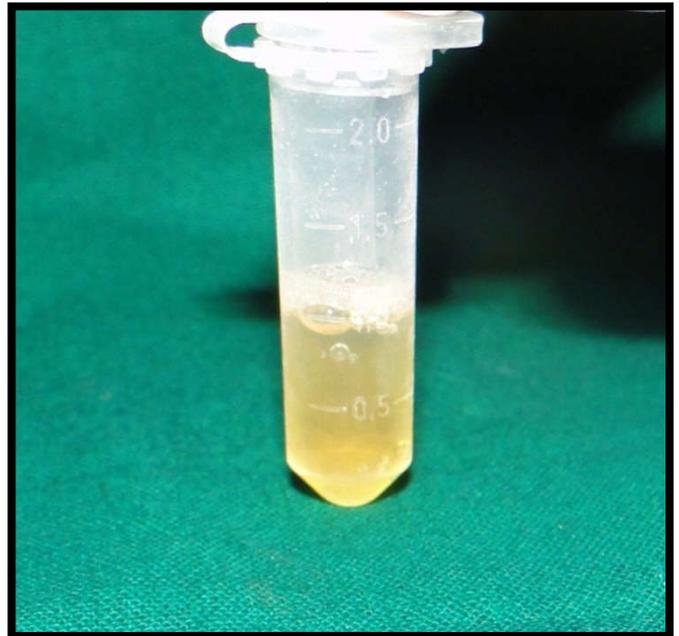


Figure 2. Plasma transferred to Eppendorf tube

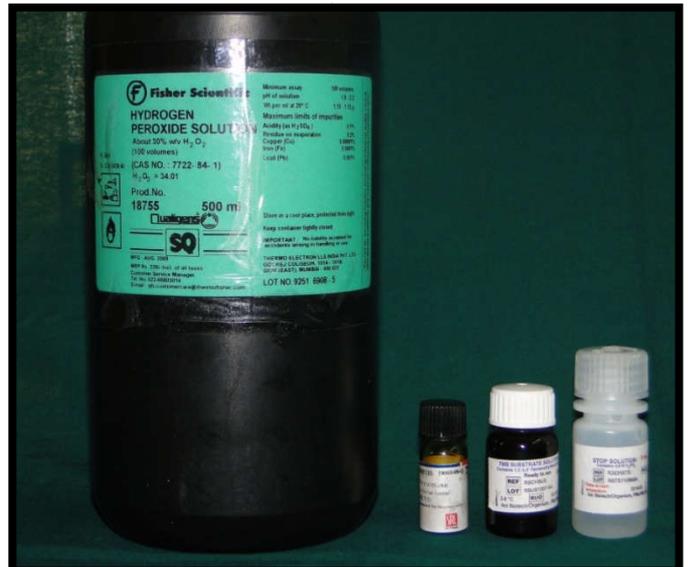


Figure 3. Reagents used

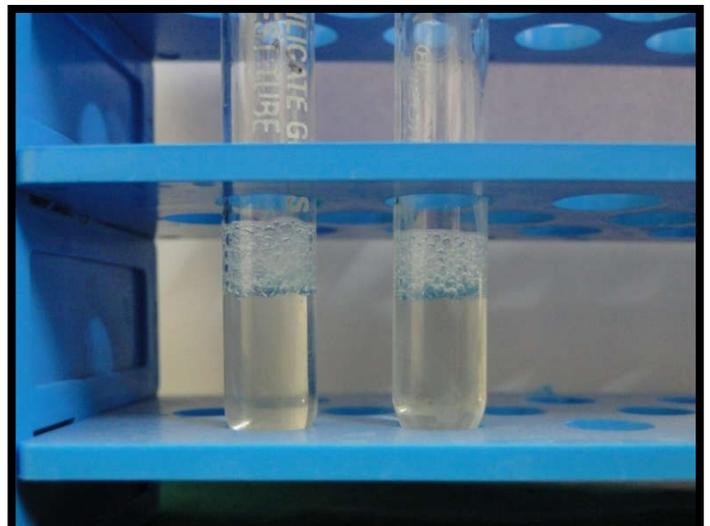


Figure 4. Plasma mixed with reagents



Figure 5. Cuvettes



Figure 6. Spectrophotometer

On comparing SBI levels using repeated measures ANOVA showed significant differences at different time points is depicted in Table 3 The probing pocket depth in test groups at baseline is 4.067 ± 0.306 , 1st month is 3.200 ± 0.200 , 2nd month 2.519 ± 0.215 is depicted in Table 2. The clinical attachment levels in test groups at baseline is 4.257 ± 0.124 , 1st month is 3.172 ± 0.295 , 2nd month 2.570 ± 0.273 is depicted in Table 2. The relationship between plasma ROM levels and clinical parameters in test group at baseline, 1st and 2nd month is shown in Table 3. At baseline plaque index was significant ($p=0.026$). The other parameters at baseline, 1st, 2nd month are non-significant.

DISCUSSION

SRP has become the “gold standard” nonsurgical treatment of periodontitis, with multiple clinical studies demonstrating that it effectively reduce the microbial load and lead to reduction in bleeding on probing and probing depth and allow for gain in clinical attachment level.^x An effective reduction of the subgingival biofilm can effectively result in reduction of plasma ROS level, which can be achieved by employing various techniques. However, important for a lasting success of

a treatment is a continuous supportive periodontal therapy employing individual prophylactic measures such as Scaling and Root Planing (SRP) and regular professional tooth cleaning. The improvement of oxidative stress in the periodontium by periodontal treatment would contribute to a reduction in circulating ROS levels.^{xi} The patients in the test group had higher plasma ROM levels (321 ± 47.54) than the control group (162.6 ± 10.5) at baseline. The higher ROM levels in patients with Chronic Periodontitis was in accordance to study done by Brock *et al.* 2004 who measured the local and systemic total concentrations of antioxidants in patients with periodontitis and periodontally healthy subjects.^{xii} In the test group, the clinical parameters like PD, CAL, SBI and PI were measured at baseline. After a meticulous SRP, plasma ROM level and the clinical parameters were measured at the first and second month. Results showed a reduction in the plasma ROM level as well as a significant reduction in the clinical parameters, 2 months after non-surgical periodontal therapy ($p<0.001$). The non-surgical periodontal therapy proved to be effective in not only improving the clinical parameters but also effectively reduced the plasma ROM levels (D'Auito *et al.*).^{xiii} The ROM levels of test group at baseline is (321 ± 47.54) and at first month (209.7 ± 29.48) and at second month ($139.3 \pm$

12.7) which was in accordance with the study done by Tamaki *et al* who reported that there was reduction in the plasma ROM level after non-surgical periodontal therapy.¹¹ However our results are contrary to the study by Kim *et al* who proposed that nonsurgical therapy do not improve the TAS in severe Chronic Periodontitis patients.^{xiv} The mean Plaque index levels in test group at baseline is 2.453 ± 0.342 , 1st month is 1.340 ± 0.250 , 2nd month 0.590 ± 0.255 was in accordance to the study done by Lavanchy *et al*.^{xv} The mean SBI levels in test groups at baseline is 2.353 ± 0.302 , 1st month is 1.473 ± 0.149 , 2nd month 0.550 ± 0.165 was in accordance to the study done by Badersten *et al* who reported marked improvement in bleeding on probing following non-surgical periodontal therapy.^{xvi}

The probing depth in test groups at baseline is 4.067 ± 0.306 , 1st month is 3.200 ± 0.200 , 2nd month 2.519 ± 0.215 . The mean reduction in the probing depth was in accordance with those of the Greenstein *et al* showing that treatment of periodontitis by SRP result in reductions in probing depth (eg, a mean reduction of 1.29 mm for 4-6 mm pockets and a mean of 2.16 mm for pockets of > 7 mm) and subgingival bacterial loads and gain in clinical attachment.^{xvii} Limitations of the present study include small sample size and changes in plasma ROM levels linked with the factors such as maintenance of oral hygiene. Long term studies with larger sample size are required to evaluate the role of non-surgical periodontal therapy in the reduction of ROS levels.

Conclusion

Our results suggest that patients with Chronic Periodontitis had higher plasma ROM level than controls. Non-surgical periodontal treatment was effective in improving clinical parameters and reducing plasma ROM level. In patients with Chronic Periodontitis, a reduction in periodontal inflammation by non-surgical periodontal treatment might be beneficial in preventing systemic disease by decreasing circulating ROS.

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