Assessment of Relative Percentage Change in Clinical Parameters and Systemic Levels of Rbc-Sod, Gpx and Vitamin-C in Patients with Chronic Periodontitis After Scaling and Root Planning

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ABSTRACT

Introduction and background: Periodontitis, an inflammatory disorder of the periodontium, has been associated with oxidative stress (OS). Recent studies have linked an abnormal OS burden in individuals with periodontitis. Dental professionals employ scaling and root planning (SRP) therapy to treat this disorder. Scarcity of literature associating the quantification of effects of SRP on periodontal parameters and systemic biochemical OS markers has provoked us to take up this study.

Aims and Objectives: To quantify the SRP therapy outcome as relative percentage change and gauge the association in the improvements in clinical parameters to those of biochemical OS markers.

Methodology: Individuals with chronic periodontitis were clinically and biochemically evaluated. SRP therapy was performed and a follow-up was done after 3 months. The mean values of study parameters were subjected to statistical analysis.

Results: Individuals with chronic periodontitis showed altered clinical and biochemical OS markers. After SRP, both clinical and biochemical parameters showed significant (p<0.05) differential improvements from their corresponding pre-treatment values. When the relative percentage was assessed, the improvements in clinical parameters were higher than that of biochemical parameters. Further the relative % change in clinical parameters showed weak correlation to that of biochemical parameters.

Conclusion: The SRP is beneficial for individuals with periodontitis. Further the improvement in clinical parameters is accompanied by improvement in biochemical OS parameters.

Keywords: Chronic periodontitis, Oxidative stress, Biochemical markers, Scaling and root planning, Relative percentage change

INTRODUCTION

Chronic periodontitis (CP) is an inflammatory disorder of the periodontium, which affects the supportive tissue of the teeth [1]. Oxidative stress (OS) has been related to the onset and or progression of growing number of human diseases [2], including periodontitis [3]. OS has been linked with both, onset of periodontal tissue destruction and progression of CP [4,5]. The role of reactive oxygen species (ROS), antioxidant (AO) systems and the products of OS play an important role in the pathology of periodontitis [1].

Scaling and root planning (SRP) is the phase I periodontal therapy and has been employed in controlling CP [6]. This therapy has yielded beneficial effect on clinical parameters like prolong depth (PD), clinical attachment level (CAL) [6], and healthy outcomes in the levels of biochemical markers like; 1L-1β, TNF-α, hsCRP, E-selectin etc. [7,8]. The

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bidirectional relationship between CP and OS is established [9]. The CP affects the OS status in the individuals and controlling the CP through the application of SRP has beneficial outcomes in OS markers [10,11]. Therefore, the present study was planned to quantify the association of the extent of improvement in clinical parameters to those of some systemic AO markers.

MATERIALS AND METHODS

Study groups

Group I (C): Individuals with slight chronic periodontitis (CAL<3 mm); n=120 (males=65, females=55), Mean age= 38.51±5.55 comprises the control group. They were apparently healthy with good oral and systemic health and have not reported any habits, any illness needing medication and/or hospitalization in the last six months. They were clinically evaluated only once, and their blood sample was collected and analyzed only once.

Group II (CP): Individuals with severe chronic periodontitis (CAL≥3 mm); n= 86 (males=51, females=35), Mean age= 40.9±4.61 formed this group. The subjects in this group were otherwise healthy, with no history of major illness and consumption of antioxidants, antibiotics, anti-inflammatory or any other drugs and had not received any periodontal therapy for at least 6 months prior to the inception of the study. Individuals having past illness and undergoing any treatment, diabetics and alcoholics were excluded from the study. This group has received SRP therapy and a follow-up was done after 3 months.

Clinical Measurements

The individuals in both the study groups were clinically evaluated for chronic periodontitis according to the criteria of the American Academy of Periodontology (1999) [12]. The periodontal status of all individuals was evaluated by measurement of gingival index (GI) [13], plaque index (PI) [14], probing depth (PD) and clinical attachment level (CAL). The PD and CAL measurements were done as prescribed [12,15]. All clinical measurements were evaluated using University of North Carolina (UNC-15) probe (Hu-Friedy, Chicago).

Sample Collection

A total of 4 ml venous blood was collected in disposable syringe from all the subjects following standard precautionary measures. Of this 2 ml blood was used for analysis of RBC-SOD (Superoxide Dismutase) and GPx (Glutathione Peroxidase) and the remaining 2 ml was allowed to stand at room temperature for 30 min and centrifuged at 3,000 rpm for 20 min to obtain serum, which was stored at −4 °C until further analysis of vitamin C. All the biochemical markers were measured on calibrated semi auto analyser BIOTRON BTR-830 (Ranbaxy laboratories, India). The blood samples were collected twice once at

baseline (C and CP group) and then after 3 months of SRP therapy (CP group).

Biochemical studies

RBC-SOD and GPx: The RBC-SOD and GPx were measured using RANSOD and RANSEL kits (Randox Laboratotries, UK) respectively following the manufacturer's instructions [16,17].

Vitamin C: The serum vitamin C content was measured using the dinitro-phenyl hydrazine (DNPH) method. Briefly, in strong acidic medium, vitamin C is oxidized to diketogluonic acid which reacts with 2,4 DNPH to form diphenylhydrazone which dissolves in strong sulphuric acid solution to produce a red colored complex which was measured at 500 nm [18].

SRP therapy: The participants in group CP received periodontal therapy, which included SRP and oral hygiene instructions. The SRP was performed by qualified periodontologist using ultrasonic instrument (Electro Medical System, Switzerland) and manual Gracey curettes (Hu-Friedy, Avco). The instructions for oral hygiene included demonstration of Bass technique of brushing [19] and instruction to brush twice daily after meals. Post SRP therapy a follow up was done after 3 months.

Relative % change calculation: Relative % change is calculated using the formula-

$$[(\text{Baseline value} - \text{Post Treatment value}) \div \text{Baseline value}] \times 100$$

Negative value indicates post treatment higher value and vice versa.

Statistical Analysis: The statistical analysis for study parameters was done using Statistical Package for Social Sciences (IBM-SPSS version 19) for MS Windows. The values were expressed as mean ± SD across the study groups. The independent sample t test was employed for the comparison of significance of difference of mean values. The correlation of relative % change in clinical and biochemical parameters was assessed using two tailed Pearson correlation tests. P value < 0.05 is considered to be statistically significant.

RESULTS

The obtained values indicate a significantly higher (p < 0.001) mean values of clinical parameters and level of RBC-SOD in individuals belonging to CP than C group. The mean values of GPx and vitamin C were significantly lowered (p < 0.001) in individuals of CP than those of C group **(Table 1)**. Furthermore, the results **(Table 2)** showed a statistically significant difference (p < 0.001) in the post treatment mean values of clinical and biochemical parameters compared to their corresponding baseline (pre-treatment) values for individuals of CP group. **Table 3** represents the mean relative % change in the clinical and biochemical parameters in CP group. Among the biochemical markers GPx and

vitamin C showed significant (p<0.05) negative correlation
to PI, PD and GI respectively **(Table 4)**.

Table 1. Comparison of Baseline Clinical Parameters and Biochemical Markers between C and CP Group.

Clinical Parameters	Mean ± SD		p* value
	Group C (n = 120)	Group CP (n = 86)	
GI	0.67 ± 0.11	2.39 ± 0.48	<0.001
PI	0.43 ±0.42	2.27 ± 0.52	<0.001
PD (mm)	1.73 ± 0.30	5.44 ± 0.44	<0.001
CAL (mm)	1.83 ± 0.30	7.68 ± 0.88	<0.001
Biochemical Oxidative Stress Markers			
RBC-SOD (U/gmHb)	290.85 ± 38.27	527.80 ± 78.06	<0.001
GPx (U/gmHb)	13.60 ± 1.43	8.27 ± 1.23	<0.001
Vit C (µM/L)	35.59 ± 3.87	25.09 ± 3.88	<0.001

*Values for mean ± SD, * p values are obtained using independent sample t test, p values <0.05 is considered to be statistically significant GI=Gingival Index, PI= Plaque Index, PD=Probing Depth, CAL=Clinical Attachment Level, SOD=Superoxide Dismutase, GPx= Glutathione Peroxidase*

Table 2. Comparison of baseline and post-treatment values of clinical parameters and biochemical markers in CP Group (n= 120).

Clinical Parameters	Mean ± SD		p* value
	Baseline	**Post-Treatment**	
GI	2.39 ± 0.48	1.47 ± 0.48	<0.001
PI	2.27 ± 0.52	1.39 ± 0.49	<0.001
PD (mm)	5.44 ± 0.44	4.65 ± 0.51	<0.001
CAL (mm)	7.68 ± 0.88	6.10 ± 1.02	<0.001
Biochemical Oxidative Stress Markers			
RBC-SOD (U/gmHb)	527.80 ± 78.06	442.80 ± 71.46	<0.001
GPx (U/gmHb)	8.27 ± 1.23	9.63 ± 1.09	<0.001
Vit C (µM/L)	25.09 ± 3.88	30.99 ± 3.73	<0.001

*Values for mean ± SD, * p values are obtained using paired sample t test, p values <0.05 is considered to be statistically significant. GI=Gingival Index, PI= Plaque Index, PD=Probing Depth, CAL=Clinical Attachment Level, SOD=Superoxide Dismutase, GPx= Glutathione Peroxidase*

DISCUSSION

The present study attempts to understand and probably quantify the relationship between the extent of improvement in clinical periodontal parameters and systemic AO marker after SRP therapy. The result of this study demonstrated that the individuals in CP group showed higher clinical damage and altered systemic AO markers then those of individuals in C group **(Table 1)**. SRP was beneficial in improving clinical as well as biochemical markers in CP group **(Table 2)**. Furthermore, the relative percentage change in clinical parameters showed some correlation with systemic AO markers **(Table 4)** although it couldn't reach statistical significance for all the parameters.

Oxidative stress (OS) has been linked with both onset of periodontal tissue destruction and systemic inflammation [4] with increased ROS concentration leading to oxidative

Table 3. Relative % change values in clinical parameters and biochemical markers in CP Group (n = 120).

Clinical Parameters	Mean ± SD Relative% Change Values
GI	38.49 ± 10.31
PI	38.76 ± 10.33
PD (mm)	14.52 ± 4.13
CAL (mm)	20.57 ± 6.07
Biochemical Markers	Mean ± SD Relative % Change Values
RBC-SOD (U/gmHb)	16.12 ± 2.85
GPx (U/gmHb)	-16.44 ± 4.62
Vit C (µM/L)	-23.57 ± 6.63

Values are mean ± SD, Relative % change is calculated using the formula [(Baseline value– Post-Treatment value)/Baseline value x100], Negative value indicates post treatment higher value and vice versa. GI=Gingival Index, PI= Plaque Index, PD=Probing Depth, CAL=Clinical Attachment Level, SOD=Superoxide Dismutase, GPx= Glutathione Peroxidase

Table 4. Correlation of Relative % Change in Clinical Parameters and Biochemical Markers after SRP in CP Group (n=120).

Clinical Prameters→ Biochemical Markers↓		GI	PI	PBI	PD	CAL
RBC-SOD (U/gmHb)	r value	.107	.079	.153	.171	.188
GPx (U/gmHb)	p value	.327	.467	.158	.115	.083
Vit C (µM/L)	r value	-.149	-.232*	-.080	-.221*	-.075
	p value	.171	.031	.466	.041	.491
	r value	-.213*	-.108	-.017	-.065	-.013
	p value	.049	.322	.876	.555	.906

*Values are represented as Pearson Correlation (r values), Negative values indicate inverse association and vice versa, p values are obtained using Pearson Correlation test, p values < 0.05 is considered to be statistically significant. *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). GI=Gingival Index, PI= Plaque Index, PD=Probing Depth, CAL=Clinical Attachment Level, SOD=Superoxide Dismutase, GPx= Glutathione Peroxidase*

damage and an impaired circulating oxidant: antioxidant balance [20,21]. All mammalian cells contain antioxidants (Enzymes, other proteins, metabolites and vitamins) that prevent or limit oxidative tissue injury caused by ROS [3].

The findings related to the pre and a post-treatment change in RBC-SOD, GPx and vitamin C has been discussed earlier by us [22]. The beneficial effects of SRP on systemic inflammatory [23,24] and OS markers [1,25] have been reported. Further the studies [26,27] have associated periodontal clinical markers to some biochemical inflammatory [26-28] and OS [5,29,30] markers. Some studies [26,27] have shown a positive association between periodontal clinical markers and IL-6, a negative association with IL-10 [31] while others [28,32] couldn't find any association between them. OS markers like GPx (5), SOD, TAOC (29) and AO potential [33] were also significantly [29] and non-significantly [33] correlated to the periodontal clinical markers. The study by Teles et al. [28] have studied relationship among IL-6, TNF-α, adipokines and vitamin D in serum of CP individuals and reported correlation among serum parameters but no association between them and clinical parameters [28].

Most of the above studies have taken into consideration the average or mean values of the study parameters for their correlation and the subsequent conclusions. However, up to our knowledge, the association between the extent of

improvement (relative% change) in the clinical and biochemical parameters has not been reported, that has been endeavored in this study. In the present study the mean relative % change values observed in the CP group for clinical parameters ranged between 14.52% to 38.76% and those for biochemical markers were between-23.51% to 16.12% **(Table 3)**. Among the biochemical parameters, GPx and Vitamin C indicated post treatment higher values compared to the baseline and hence negative values for relative % changes. RBC-SOD and all the clinical parameters showed post treatment lowered values hence positive values for the relative % change. The correlation between clinical parameters and biochemical markers has been displayed in **Table 4**. The obtained results of this study showed an insignificant ($p \geq 0.05$) positive weak association for RBC-SOD with the clinical parameters. The GPx and vitamin C have showed significant ($p \leq 0.05$) correlation to PI, PD and GI respectively. Both the biochemical markers (GPx and vitamin C) showed weak negative correlation to other clinical parameters and the values could not reach statistical significance ($p \geq 0.05$). Furthermore as demonstrated in the results, among the clinical parameters, PD and CAL relative % change values(14.52% and 20.57% respectively) were lower than those of GI and PI (38.49% and 38.76% respectively). This indicates that SRP and oral hygiene maintenance may prevent plaque and inflammation, but the therapy regime may contribute to lesser extend in periodontal tissue building and gaining attachment level, at least through the conditions prevailed in this study. Oral clinical improvement is reflected in systemic improvement, however compared to clinical parameter biochemical markers showed relatively lowered relative % change values (-23.51% to 16.12% v/s 14.52% to 38.76% **(Table 3)**. This finding may be attributed to the time period of the study (a follow-up after3 months period) as biochemical markers may need longer time to show the equivalent improvement as those of clinical parameters. Furthermore, biochemical markers are in a dynamic state and may also be affected by other physiological conditions, the monitoring of those variations was beyond the scope of the present study. Albeit varying response, it is promising to note that SRP was effective in improving clinical as well as biochemical markers compared to their corresponding baseline values. Also, the extent of improvement (the relative % change) in clinical parameters showed some correlation to the improvement in biochemical markers, although the correlation could not reach statistical significance for all the parameters.

CONCLUSION

The present study has showed favorable effects of SRP on clinical and biochemical parameters for individuals with chronic periodontitis. The study findings may encourage dental professionals to include SRP as a beneficial tool in their therapy regime not only to lower local inflammation in individuals with periodontitis but also in improving the systemic health of the diseased individuals.

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