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## Comparative evaluation of erythrocyte superoxide dismutase levels in chronic periodontitis patients before and after periodontal therapy

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### Abstract

**Background:** Only a few studies have examined the association between periodontitis and erythrocyte superoxide dismutase level. The aim of this study was to compare erythrocyte superoxide dismutase levels in subjects with and without periodontitis, before and after non-surgical periodontal therapy.

**Methods:** This comparative study was done on subjects  $\geq 30$  to  $\leq 60$  years old. Group A consisted of 20 subjects without periodontitis, and Group B consisted of 20 subjects with periodontitis. Body mass indexes (BMI) and clinical parameters, including oral hygiene index-simplified (OHI-S), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), haemoglobin level and erythrocyte superoxide dismutase level, of all subjects were recorded. All subjects received non-surgical periodontal therapy (scaling and root planing). After three months, all subjects were reexamined and clinical parameters and erythrocyte SOD levels were evaluated and compared with the baseline values.

**Results:** There were significant differences between Group A and Group B in regard to baseline levels of OHI-S, GI, PPD, and erythrocyte SOD ( $p < 0.05$ ). There was no clinical attachment loss in Group A, either at baseline or after three months. At the end of three months, Group B showed improvement in all clinical parameters ( $p < 0.05$ ), and their erythrocyte superoxide dismutase levels also significantly increased ( $p < 0.05$ ), although the values never reached those without periodontitis.

**Conclusion:** The erythrocyte superoxide Dismutase levels of subjects with periodontitis (Group B) significantly increased three months after non-surgical periodontal therapy, although they never reached the same level as that of the subjects without periodontitis (Group A).

**Keywords:** Erythrocyte superoxide dismutase, Non-surgical periodontal therapy, Periodontitis

### Introduction

When stimulated by periodontopathic bacteria, host cells (e.g. polymorphonuclear leukocytes) release reactive oxygen species (ROS) as part of the immune response [1]. Reactive oxygen species (ROS) play crucial roles in normal physiological processes and it represents an important pathogenic mechanism for tissue damage and diseases associated with phagocytic infiltration [2]. Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, nitrous oxide and hydrogen peroxide, are produced via the bacteria-host mediated pathway inducing tissue break down. The removal of ROS by antioxidant defence systems is essential for healthy aerobic life. In healthy organisms, the balance is maintained by the interaction of oxidants and antioxidants whereas, under various pathological conditions, the balance may be shifted towards the oxidative side [3, 4].

Preventive antioxidants suppress the formation of free radicals, for example, enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) and metal ion sequestrators such as albumin, lactoferrin, transferrin, and haptoglobin [5]. Scavenging (chain-breaking) antioxidants scavenge radicals to inhibit the chain initiation and break chain propagation for example, vitamin C, vitamin A, vitamin E, and uric acid [5].

Oxidative stress is "a disturbance in the pro-oxidant-antioxidant balance in favour of the former, leading to potential damage" [6]. Smaller changes in redox state-trigger gene transcription events lead to tissue damage secondary to induction of proinflammatory state [7].

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Larger upward shift in the pro oxidant /antioxidant ratio bring direct damage to vital biomolecules and structures [7]. Within mammalian tissues, the most significant antioxidant is perhaps superoxide dismutase (SOD), which catalyses the dismutation of superoxide anion ( $O_2^-$ ) to continuously form  $H_2O_2$  and  $O_2$ . Together with Catalase and Glutathione peroxidase, they provide protective mechanisms against the intracellular accumulation of  $H_2O_2$ . These enzymes accelerate  $H_2O_2$  reduction to water [7].

Fridovich and McCord discovered the activity of superoxide dismutase. Three forms of superoxide dismutase are present in human. SOD concentrations have been estimated in relation to periodontitis in various body fluids, with inconclusive results [8, 9, 10]. Therefore there is a need for careful assessment of SOD levels and their association with periodontitis.

According to Ramamurthy NS *et al.* [11] ROS directly cause periodontal tissue damage by the degradation of the extracellular matrix components of periodontal tissues or play an indirect role in potentiating extracellular matrix degradation by matrix metalloproteinase, via the activation of latent enzymes, such as collagenase and gelatinase. Here the role of SOD is very important in limiting the progression of the disease process [11]. This is another reason why we want to determine the levels of SOD in our population. Biochemical analysis showed that the human periodontal ligament contained about twice as much SOD activity as human skin, but lower than red blood cells. The concentrations were positively or negatively related to probing depth (PD) and had a definite correlation with the progression of periodontal disease [20]

The authors hypothesized that erythrocyte SOD level in individuals without periodontitis will be higher than those of individuals with periodontitis, and that non-surgical periodontal therapy can increase the levels of SOD in individuals with periodontitis.

Developing newer dental products that incorporates antioxidant micronutrients in Local Drug delivery and oral mouth rinses can nourish and protect the periodontium better. So it is essential to study the protective role of antioxidants against free radicals.

This study compared the effectiveness of non-surgical periodontal therapy on erythrocyte Superoxide dismutase levels in subjects with and without periodontitis; based on which we intend to assess the relationship between periodontitis and erythrocyte superoxide dismutase.

## Materials and Method

### Study Setting

This study was conducted in the Outpatient Department of Periodontics, Amrita School of Dentistry, Kochi. and was approved by the ethical committee of the Amrita School of Dentistry India.

### Sample Size Estimation

The sample size to ensure adequate power for this study was calculated. Based on the values, it was found that 15 subjects per group were necessary to provide 80% power at a 95% confidence interval ( $\alpha = 0.05$ ). Based on the inclusion and exclusion criteria subjects were divided into two groups; Group A [systemically healthy subjects without periodontitis] and Group B [systemically healthy subjects with periodontitis]

## Criteria for selection

### Inclusion criteria

#### Group A (subjects without periodontitis)

1) Age  $\geq 30$  and  $\leq 60$  years; 2) systemically healthy 3) no probing pocket depth  $> 3$ mm, no clinical attachment loss; 4) the presence of more than 20 natural teeth; and 5) No blood dyscrasia 5) haemoglobin level within normal limits.

**Group B (subjects with periodontitis)**- The study participants were the subjects with age  $\geq 30$  and  $\leq 60$  years, systemically healthy; clinical attachment loss  $\geq 3$ mm and in 5 or more teeth and presence of more than 20 number of natural teeth, haemoglobin level within normal limits.

Exclusion criteria for both groups were:

- 1) history of any systemic illness, history of periodontal treatment (scaling and root planing within 6 months),
- 2) history of antibiotic and antioxidant vitamin use within the past 6 months
- 3) patients with recent significant blood loss or bleeding disorders
- 4) hepatitis or human immunodeficiency virus (HIV) infection and immunosuppressive chemotherapy
- 5) pregnancy or lactation
- 6) smoking or alcoholism.

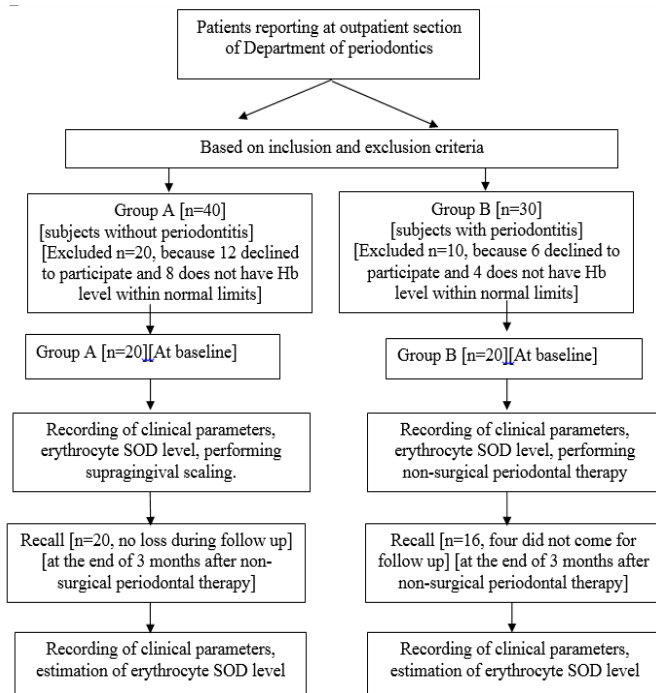
### Study Design and Study Participants

In this cross sectional study, the participants were healthy subjects  $\geq 30$  to  $\leq 60$  years old with and without periodontitis who reported to the Outpatient Department of Periodontics, Amrita School of Dentistry. Subjects were consecutively selected; randomization was not done due to the limited availability of the subjects who fulfilled the criteria for selection.

Subjects who fulfilled the inclusion/exclusion criteria were invited to participate in the study. Subjects were divided into two groups, Group A [subjects without periodontitis] and Group B [subjects with periodontitis], based on the inclusion and exclusion criteria.

All subjects were informed of the nature, potential risks, and benefits of the study, and they were given a participant information sheet in the local language (Malayalam) with a brief description of the purpose and process of the study. A written informed consent was obtained from the subjects who agreed to participate in the study. Those who declined to provide their consent were excluded.

A total of 70 subjects were found to be eligible for participation in this study. 40 subjects met the criteria for Group A [subjects without periodontitis], 30 subjects met the criteria for Group B [subjects with periodontitis]. Twenty subjects in Group A and 10 subjects in Group B were excluded because 18 declined to participate in the study, while 12 does not have Hb level within normal limits. leaving a total of 40 subjects, with 20 subjects in each group (Figure 1). Even though 15 subjects per group were necessary to provide 80% power at a 95% confidence interval, the study was conducted with 20 subjects in each group, thereby anticipating participant loss during follow-up.



**Fig 1:** Flowchart showing the study design and flow of participants through the study.

### General Evaluation

All subjects were confirmed by measuring their Hb levels using SAHLIS haemoglobinometer. All subjects had Hb levels within normal limits. The weight of the subjects, in kilograms, and their height, in meters, were recorded using a weighing machine and metric tape respectively. The body mass index (BMI) was calculated using the formula:  $BMI = \frac{\text{weight (kg)}}{[\text{height (m)}]^2}$

### Periodontal Examination

Each subject underwent a full mouth periodontal probing and charting by one trained periodontist. The following parameters were analyzed: 1. Oral hygiene index – Simplified (OHI-S) [12], 2. Gingival index (GI) [13], 3. Probing pocket depth (PPD) [14], 4. Clinical attachment level (CAL) [14]. PPD and CAL were measured using the University of Michigan “O” probe with Williams markings at six sites per tooth (mesiobuccal/labial, midbuccal/labial, distobuccal/labial, mesiolingual/palatal, midlingual/palatal, and distolingual/palatal) in all teeth, excluding third molars. To ensure reproducibility during the examinations, a customized acrylic stent was used as a reference to determine the site and angle of measurement.

### Non-Surgical Periodontal Therapy

After recording clinical parameters and collecting blood samples, all subjects received non-surgical periodontal therapy.

The supragingival debridement was done for Group A and SRP was done in group B. It was performed by one trained periodontist in two sessions (morning and afternoon sessions) within 24 hours, using Gracey curettes. After the SRP sessions, all subjects received oral hygiene instructions for home care (tooth brushing technique and interdental cleaning) from the same trained periodontist.

### Recall Visit

All subjects were recalled finally at the end of three months and their erythrocyte SOD levels and all other clinical parameters were evaluated.

## Biochemical Analysis

### Evaluation of SOD

To estimate erythrocyte SOD level, 2 ml of peripheral venous blood was collected in a violet vacutainer, which contained the anticoagulant ethylenediaminetetraacetic acid (EDTA), from the antecubital vein of each subject by a trained nurse.

The haemoglobin status of all subjects was confirmed by measuring their haemoglobin level using haematocrit method. All subjects had their Hb level within normal limits.

### Sample collection

The blood samples for erythrocyte SOD were collected in EDTA vacutainers (2 ml each). The plasma was separated and blood cells were used for hemolysate preparation. The blood cells after separation of the plasma were washed with 4 ml of cold normal saline (0.9% NaCl) centrifuged at 3000rpm for 15 minutes and supernatant discarded. This was repeated for 2 or more times. The washed packed cells were hemolysed with 30 ml ice cold distilled water and refrigerated. SOD was estimated by the inhibition of auto oxidation of pyrogallol according to the procedure described by Marklund<sup>15</sup>.

### Estimation of superoxide dismutase (E.C 1.15.1.1) activity

#### Reagents

- 50 mM Tris HCL buffer
- 0.2 mM pyrogallol
- 1mM DTPA (Diethyl Triamine Penta Acetic Acid) buffered substrate : to 50 ml buffer add 0.00126gm pyrogallol and 0.0196 gm DTPA

#### Procedure [15, 16]

2 ml buffered substrate is taken as the reagent blank. Readings are noted. Four sample readings are taken at 10 seconds interval at 420 nm (double beam spectrophotometer) by adding 100 µl sample to 2ml buffered substrate. The super oxide dismutase activity was measured by the inhibition of auto oxidation of 0.2 mM pyrogallol in 50mM Tris Hcl buffer containing 1mM DTPA. The percentage inhibition of rate of auto oxidation of pyrogallol was initiated by addition of 50µl of hemolysate to 1ml of buffered substrate in a cuvette. Values are expressed in U/gm Hb

#### Calculation

$$\frac{\text{Rate of oxidation of sample} \times 50 (\text{percentage inhibition}) \times 10 (\text{dilution})}{\text{Rate of oxidation of control}} = \text{U/gm Hb}$$

#### Statistical Analysis

Statistical tests were performed using software IBM-SPSS [Statistical Package for Social Sciences, version 20]. Mean age, body mass index [BMI], probing pocket depth [PPD], clinical attachment level [CAL], gingival index [GI], oral hygiene index-simplified [OHI-S] and erythrocyte SOD level were calculated per group. Inter group comparison of age and BMI was done using Independent Sample t test, while that of gender was done using Chi Squared test. Karl Pearson Coefficient of correlation was used to test correlation of age and body mass index [BMI] with SOD levels. Intra group and inter group comparison of parameters at baseline and at the end of 3 months recall interval was done using Paired t test and Independent Sample t test respectively. The results were considered statistically significant when  $p$  value was  $<0.05$ .

#### Results

Group A consisted of 10 males (50%) and 10 females (50%), with a mean age of  $38.6 \pm 7.34$  years and a mean BMI of 24.2

$\pm 1.92 \text{ kg/m}^2$ . Group B consisted of 10 males (50%) and 10 females (50%), with a mean age of  $41.8 \pm 8.5$  years and a mean BMI of  $24.7 \pm 1.94$ . The two groups were similar in terms of gender, BMI and age ( $P > 0.05$ ) (Table 1). The p-value in group A is less than the significance level 0.05 ( $p < 0.05$ ); the Pearson correlation between age and SOD level is significant in group A. The p-value in group B is greater than the significance level 0.05; the correlation between age and SOD level is not significant in group B. There was no correlation between BMI ( $p > 0.05$ ) with SOD level in either group.

**Comparison of Parameters at the Baseline**

At the baseline, Group B had significantly higher OHI-S ( $p < 0.05$ ) (Table 1) (Figure 2A), GI ( $p < 0.05$ ) (Table 1) (Figure 2B), and PPD ( $p < 0.05$ ) (Table 1) (Figure 3) levels than Group A. Group B had a CAL of  $5.738 \pm 0.49$  mm (Table 1) (Figure 3) at the baseline, while Group A had no clinical attachment loss.

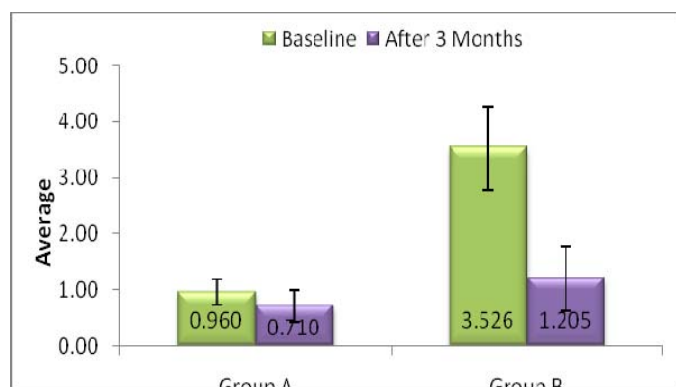
At the baseline, the SOD level of Group B ( $12.24 \pm 6.32 \text{ gm/Hb}$ ) was less than that of Group A ( $39.50 \pm 9.710 \text{ Gm/Hb}$ ), and the difference was statistically significant ( $p < 0.05$ ) (Table 1) (Figure 4).

**Post-Intervention Comparison of Parameters**

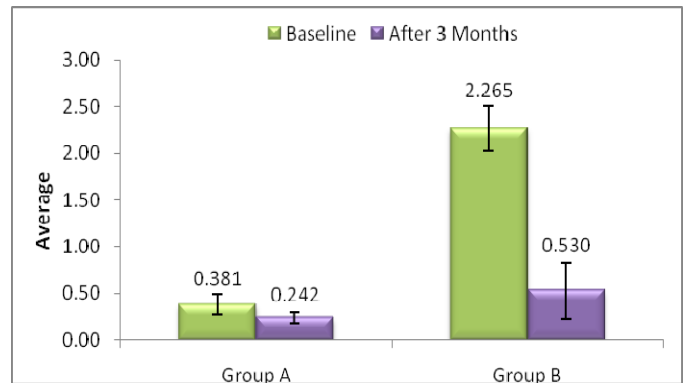
In Group B, at the end of three months after SRP, there were statistically significant reductions in OHI-S ( $p < 0.05$ ) (Table 1) (Figure 2A), Gingival Index ( $p < 0.05$ ) (Table 1) (Figure 2B), PPD ( $p < 0.05$ ) (Table 1) (Figure 3), CAL ( $p < 0.05$ ) (Table 1) (Figure 3) and increase in SOD ( $p < 0.05$ ) (Table 1) (Figure 4) levels.

Group A had no clinical attachment loss at the end of three months after SRP. The CAL of Group B decreased to  $3.606 \pm 0.607$  mm at the end of three months after SRP (Table 1) (Figure 3). This result indicated that even though SRP resulted in reduction in the PPD and CAL of Group B, a complete elimination of periodontal pockets and a complete gain in the clinical attachment level could not be obtained.

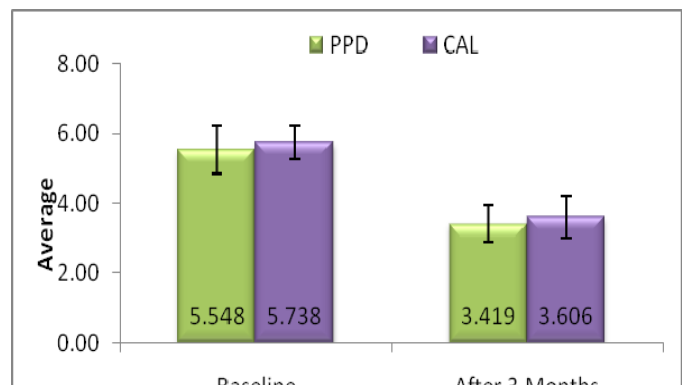
The erythrocyte SOD level of Group A at the end of three months after SRP was  $41.70 \pm 8.211$ , while that of Group B was  $24.77 \pm 10.07$ . Although the difference in the SOD levels was less compared to the difference at the baseline, it was statistically significant ( $p < 0.05$ ) (Table 1) (Figure 4). This indicated that even though there was a significant increase in SOD levels in subjects with periodontitis after SRP, the values never reached those of the healthy subjects.



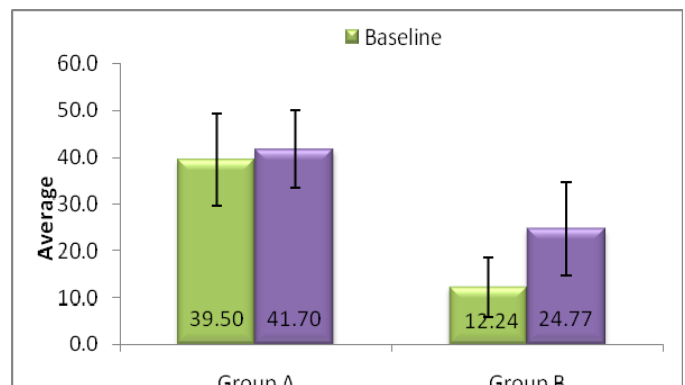
**Fig 2: A)** Comparison of OHI-S (mean  $\pm$  SD) of group A and group B at baseline and at the end of 3 months. At baseline, the OHI-S score of group B was significantly higher than that of group A (independent sample t test, \*  $P < 0.05$ ). Three months after non-surgical periodontal therapy, the OHI-S score of group B showed significant reduction (paired t test, †  $P < 0.05$ ).



**B)** Comparison of GI (mean  $\pm$  SD) of group A and group B at baseline and at the end of 3 months. At baseline, the GI score of group B was significantly higher than that of group A (independent sample t test, ‡  $P < 0.05$ ). Three months after non-surgical periodontal therapy, the GI score of group B showed significant reduction (paired t test, §  $P < 0.05$ ).



**Fig 3:** Comparison of PD (mean  $\pm$  SD) and CAL (mean  $\pm$  SD) of group B at baseline and at the end of 3 months. Three months after non-surgical periodontal therapy, group B showed significant reduction in PD (paired t test, \*  $P < 0.05$ ) and CAL (paired t test, †  $P < 0.05$ ).



**Fig 4:** Comparison of erythrocyte SOD levels (mean  $\pm$  SD) of group A and group B at baseline and at the end of 3 months. At baseline, erythrocyte SOD levels of group B were significantly lower than those of group A (independent-sample t test, \*  $P < 0.05$ ). Three months after non-surgical periodontal therapy, SOD levels of group B significantly increased (paired t test, †  $P < 0.05$ ), although the values never reached those of group A (independent-sample t test,  $P < 0.05$ ).

**Discussion**

Superoxide dismutase being one of the main antioxidant enzyme in the cell removes damaging ROS from cellular environment by catalyzing the dismutation of superoxide radicals to  $\text{H}_2\text{O}_2$  and hence plays a key role in tissue integrity. Periodontitis increases the levels of reactive oxygen species. Many studies have given inconclusive reports about the change in the level of SOD in periodontal disease. Akalin *et al.*

2009 [10], Baltacioqlu E *et al.* [17], Singh *et al.* [18], Kim *et al.* [19], Bennet H Jacoby *et al.* [20], Parth [21], Prandnya Shree Dhotre *et al.* [22] reported decreased SOD level, while Aziz *et al.* 2012 [23], Panjamurthy *et al.* 2005 [24], Wei *et al.* [25], Kawamura *et al.* [26], Akalin FA *et al.* [8], Sumitra *et al.* [16] reported increased SOD level in body fluids.

Red blood cells have the greatest SOD activity when compared to periodontal ligament and human skin [20]. It is also known that SODs have only minor activity in extra cellular fluids [20, 27]. Hence in our study we intend to find out the levels of erythrocyte SOD in subjects with and without periodontitis before and after sub gingival debridement.

In our study we used a manual method based on the ability of enzyme to inhibit the autooxidation of pyrogallol in the presence of DTPA. In this reaction there is involvement of 99% of superoxide anion radical produced, indicating an almost total participation [15]. A significant positive correlation between the salivary and the serum SOD activity was observed in the iron deficiency anaemia group [28] which is also associated with enhanced oxidative stress [29]. In our study all the subjects have haemoglobin level within normal limits which was recorded at baseline.

The reduced level of antioxidant level in diseased state in our study is in consistent with other studies in literature. Baltacioqlu E *et al.* [17], Singh *et al.* [18], Ellis *et al.*, [30] Matthews *et al.* [31] F A Akalin *et al.* [10], Ellis *et al.* [30] found a significant and progressive reduction in serum SOD activity in chronic periodontitis patients. According to Baltacioqlu *et al.* the level of SOD in serum was decreased because, SOD being a radical scavenging antioxidant is sacrificially decreased in extracellular fluids under conditions of oxidative stress even though tissue levels of SOD might have been up regulated to combat the oxidative stress. Matthews JB *et al.* [31] Singh *et al.* [18] Souvik Chakraborty *et al.* [29] found a decrease in serum SOD level in patients suffering from chronic periodontitis due to compromised oxidant antioxidant balance.

Parth *et al.* [21] also reported decreased erythrocyte SOD level which is in harmony with our results. According to him, in periodontitis, primary acute host defense cells (PMNS) are activated as a two stage process. Upon encountering perio pathogens (P. gingivalis, A. actinomycetemcomitans) and their antigens, present in gingival sulcus or in the blood stream, consisting of priming and full scale activation. Primed neutrophils demonstrate elevated level of ROS which shifts the said ecological balance in favour of pro-oxidant species leading to increased utilization of SOD. The Estimation of SOD was done as per MC Coord and Fridovich 1969 while we analysed as per Stefan Marklund [15].

In contrast to our study kale *et al.* [32] and Aziz *et al.* [23] examined erythrocyte SOD concentrations and found to be significantly higher in the CP group compared with the control group. According to them this enhanced activities of SOD in erythrocytes could be related to a greater degree of oxidative stress in patients with chronic periodontitis. Here the erythrocyte SOD activity was measured by Ransod kit.

N. Unni C *et al.* [16] estimated erythrocyte SOD levels in patients diagnosed with Polycystic Ovarian syndrome to healthy controls. She concluded that the raised erythrocyte SOD might be an over expression of the enzyme as a compensatory adaptive response for the increased demand for dismutation of superoxide radical formed in diseased condition. Here they used the Marklund [15] as in our study.

Non-surgical periodontal treatment in the form of SRP in subjects with periodontitis resulted in significant improvement in clinical as well as biochemical parameters (Table 1). The

improvement in erythrocyte SOD levels can be attributed to reduction in the levels of inflammatory cytokines due to scaling and root planing. These results are in accordance with those of Kim *et al.* [19] who also observed significant improvement in SOD level in saliva.

**Table 1:** Characteristics of Study Population and Monitored Parameters [mean  $\pm$  SD] at Baseline and 3 Months after Periodontal Therapy

Variable	Group A	Group B	p value*
Male (n)	10	10	1.000
Female (n)	10	10	
Age			
Pearson Correlation with sod at baseline	38.6 $\pm$ 7.34	41.8 $\pm$ 8.52	0.211
p value†	0.507 0.022	0.052 0.828	
BMI			
Pearson Correlation with sod at baseline	24.2 $\pm$ 1.92	24.7 $\pm$ 1.94	0.392
p value†	0.275 0.240	-0.253 0.281	
OHI-S			
Baseline 3 months p value‡	0.960 $\pm$ 0.223 0.710 $\pm$ 0.283 0.002	3.526 $\pm$ 0.745 1.205 $\pm$ 0.578 0.000	0.000 0.002
GI			
Baseline 3 months p value‡	0.381 $\pm$ 0.114 0.242 $\pm$ 0.057 0.000	2.265 $\pm$ 0.244 0.530 $\pm$ 0.299 0.000	0.000 0.000
PD (mm)			
Baseline 3 months p value‡	2.075 $\pm$ 0.240 1.830 $\pm$ 0.369 0.000	5.548 $\pm$ 0.695 3.419 $\pm$ 0.538 0.000	0.000 0.000
CAL (mm)			
Baseline 3 months p value‡	.000 000	5.738 $\pm$ 0.499 3.606 $\pm$ 0.607 0.000	0.000 0.000
SOD (Gm/Hb )			
Baseline 3 months p value‡	39.50 $\pm$ 9.710 41.70 $\pm$ 8.211 0.002	12.24 $\pm$ 6.362 24.77 $\pm$ 10.07 0.000	0.000 0.000

\* Intergroup (horizontal) comparison.

† P value of correlation.

‡ Intragroup (vertical) comparison

There was a statistically significant difference between the two groups before and after non surgical periodontal therapy. Here the erythrocyte SOD level in the periodontitis group was low in the beginning prior to therapy but increased three months after NSPT. This was in accordance with the studies by Parth Purwar *et al.* [21] and Singh *et al.* [18] who reported significant improvement in serum and salivary SOD activity at 3 months after NSPT and Sukhtankar *et al.* [33] also reported improvement via NSPT in the levels of respective antioxidant studied.

SOD levels in the periodontitis group after therapy had increased considerably but it never reached the level of subjects without periodontitis. This was in accordance with studies done by ParthPurwar *et al.* [21] and Singh *et al.* [18], Chapple *et al.* [28] suggested that non-surgical therapy with improvements in clinical parameters can increase the antioxidant defence in chronic periodontitis patients. This change in antioxidant level in erythrocytes in our study signifies the evidence that non-surgical periodontal therapy (NSPT) reduces oxidative stress both locally and systemically. This can be due to decreased level of periodontal pathogens post NSPT thereby decreasing the inflammation, which in turn reduces the reactive oxygen species (ROS) production and enabling the body defense to restore the physiologic pro-antioxidant balance. Thus Successful NSPT is highly effective in restoring super oxide dismutase level.

Non-surgical periodontal therapy cannot bring about complete elimination of all sub gingival bacteria; re-colonization can

develop over a period of time. In this study recall evaluation was done three months after the non-surgical periodontal therapy; due to time constraints we were not able to evaluate whether effects of treatment lasted beyond this time period. This can be a limitation of our study. Strengths of this study include the use of an assay where there is almost complete involvement of superoxide anion radical produced due to oxidative stress. Erythrocyte SOD levels were estimated since it was already stated that red blood cells have the greatest SOD activity as compared to periodontal ligament and it is also known that SODs have only minor activity in extra cellular fluids. Specific activity of SOD in erythrocyte was estimated by determining the Hb levels of each subject. Even though smokers were excluded from this study, demographic information and blood pressure of the subjects were not recorded which can be a limitation of this study. This study indicates that periodontitis can be considered as a, independent risk factor for antioxidant level in our body.

### Conclusion

Based on the results obtained from this comparative study, it can be concluded that at baseline erythrocyte SOD levels of subjects with periodontitis were significantly lower than that of subjects without periodontitis. At the end of 3 months after non-surgical periodontal therapy, subjects with periodontitis showed significant improvement in their periodontal health, even though complete elimination of periodontal pockets and complete gain in clinical attachment could not be achieved. Erythrocyte SOD levels of subjects with periodontitis (Group B) showed significant rise at the end of 3 months after non-surgical periodontal therapy, though the levels did rise to the level of subjects without periodontitis (Group A).

Interpretations of results lead to the conclusion that oxidative stress developed due to periodontal tissue damage could have influenced the level of antioxidant enzyme in red blood cells. It indicates that periodontitis can be one of the factors for decreased antioxidant level in our body.

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