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Study to Evaluate the Effects of Periodontitis and Type II Diabetes on Serum and Salivary Superoxide Dismutase Levels

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Research Article

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ABSTRACT

Objectives: 1. Estimation of SOD levels in saliva and serum of a) Healthy patients b) Patients with chronic periodontitis c) Patients with type-II diabetes mellitus & d) Patients with type-II diabetes mellitus along with chronic periodontitis. 2. To evaluate changes in SOD levels in saliva and serum after SRP in above four mentioned groups.

Materials and Methods: This study was carried out on 40 subjects selected by random sampling method from the Outpatient Department of Periodontology, B.V.D.U Dental College and Hospital, Navi Mumbai. Study was approved by the institutional ethical committee. The selected subjects were divided into 4 groups as, Group I: Control group of ten Patients, Group II: Ten Patients with chronic periodontitis and diabetes mellitus (type II), Group III: Ten patients with chronic periodontitis and Group IV: Ten Periodontally healthy patients with diabetes mellitus (type II). Periodontal parameters, i.e. plaque index, gingival index, probing pocket depth and clinical attachment level were assessed at baseline. Blood and saliva samples were collected at baseline and on 10th day after SRP. SOD levels were estimated by using ELISA kit. Statistical analysis was done by ANOVA test.

Results: SOD concentration was higher in Group II compared to other groups. After non surgical therapy there was significant decrease in SOD concentration in all groups. ($P < 0.05$)

Conclusion: This study shows an additive effect of diabetes and chronic periodontitis on the oxidative stress. It can be concluded that SRP is helpful to decrease the oxidative stress in periodontitis and diabetes.

INTRODUCTION

The human periodontal diseases are inflammatory disorders that give rise to tissue damage and loss, as a result of the complex interaction between pathogenic bacteria and host's immune response. While the primary etiologic agent is dental plaque, the majority of periodontal tissue destruction is caused by an inappropriate host response to the micro-organisms and their products. More specifically, a loss of homeostatic balance between proteolytic enzymes (e.g. neutrophil elastase) and their inhibitors (e.g. α_1 antitrypsin), reactive oxygen species (ROS) and the antioxidant defense systems that protect and repair vital tissue, cell and molecular components is believed to be responsible for periodontal tissue destruction.

Free radicals are "species capable of independent existence that contain one or more unpaired electrons". The unpaired electrons of free radicals confer an inherent instability and high reactivity potential with other bio-molecules. ROS is a term collectively describing oxygen radicals and other non-radical but reactive oxygen derivatives, many of which are found in living organisms. ROS are continuously generated in the body during mitochondrial oxidative metabolism. In addition, ROS are generated by the inflammatory cells and other cell types. In health, it is now known that ROS, in addition to their bactericidal function, play

an important role in normal homeostasis by controlling gene expression, cellular signal transduction and maintaining vascular health.

When antioxidant defense systems are compromised or ROS-production is excessive, a state of “oxidative stress” arises and this state is an important contributing factor to tissue damage in many chronic human diseases such as chronic periodontitis and diabetes.² Oxidative stress has been linked with onset of periodontal tissue destruction and systemic inflammation. It was therefore hypothesized an association between oxidative stress and systemic inflammation in people with severe periodontitis ^[1].

Diabetes mellitus, a common metabolic disorder resulting from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria. During diabetes, persistent hyperglycemia causes increased production of free radicals especially ROS, for all tissues from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by antioxidants ^[2].

Periodontitis is an inflammatory disease of the periodontium, which affects the supporting tissues of the teeth. The imbalance in the interactions between the pathogens and host defense results in periodontal tissue breakdown. Specific bacteria induce the release of cytokines which increase the number of defense cells and their activation. ROS, such as superoxide anion, hydroxyl radical, nitrous oxide and hydrogen peroxide, are produced via the bacteria-host mediated pathway inducing tissue breakdown. At the same time, polymorphonuclear leukocytes produce and increase the concentration of ROS, resulting in oxidative damage to the periodontal tissue. Antioxidants, many of which are released locally at sites of inflammation by polymorphonuclear leukocytes (PMNLs) and /or other cells, can provide protection against ROS.

In healthy organisms, the balance is maintained by the interaction of oxidants and antioxidants whereas, under pathological conditions, the balance may be directed towards the oxidative stress ^[3]. Periodontitis is a common chronic inflammatory disease initiated by bacteria which has an increased prevalence and severity in patients with type II diabetes. Recent studies indicate that presence of periodontitis can in turn, adversely affect diabetic status and the treatment of periodontitis can lead to improved metabolic control in diabetes patients. Current evidence points to bi-directional inter-relationship between diabetes and inflammatory periodontitis. The importance of oxidative stress-inflammatory pathways in the pathogenesis of type II diabetes and periodontitis has recently received attention ^[4].

SOD is one of the most significant antioxidant within mammalian tissues which catalyses the dismutation of superoxide (O₂), an oxygen radical that is released in inflammatory pathways which causes connective tissue breakdown. This enzyme is released as a homeostatic mechanism to protect the tissues. It can be detected in extra and intra cellular compartments. SOD has been localized in human periodontal ligament, and it may represent an important defense within gingival fibroblast against superoxide release ^[4].

As described earlier there is presence of oxidative stress in both condition, i.e. in periodontitis and diabetes. According to literature these both condition are interrelated to each other, this study was aimed to evaluate the effect of these both condition on the serum and salivary levels of SOD.

MATERIALS AND METHODS

The present study was carried out on 40 subjects with age group of 30years and above selected by selective sampling method from the Outpatient Department of Periodontology, Bharati Vidyapeeth Deemed University Dental College and Hospital, Navi Mumbai. The research protocol was initially submitted to the institutional ethical committee and review board and after ethical approval, all subjects were verbally informed and written informed consent was taken for participation in the study.

All potential participants were explained about the need and design of the study. Only those subjects who could fulfill all the defined criteria and consented for the study were included. The selected subjects were grouped into four groups as follows:

Group I: Control group of ten Patients

Group II: Ten Patients with chronic periodontitis and diabetes mellitus (type II)

Group III: Ten patients with chronic periodontitis

Group IV: Ten Periodontally healthy patients with diabetes mellitus (type II).

INCLUSION CRITERIA

Group I: Periodontally and systemically healthy subjects with no history of periodontal treatment within a 3months period before enrollment in to the study.

Group II: Subjects with a diagnosis of chronic periodontitis – more than 30% sites involved, with bleeding on probing, clinical attachment loss > 5mm. and history of diabetes mellitus (type II) since more than minimum 5years

Group III: Subjects with a diagnosis of chronic periodontitis – more than 30% sites involved, with bleeding on probing, clinical attachment loss > 5mm and without any systemic disease within a 3months period before the study began.

Group IV: Periodontally healthy subjects without any systemic disease except diabetes mellitus (type II) since more than 5years.

EXCLUSION CRITERIA

1. Subjects with any other systemic disease (except Type II diabetes).
2. Subjects who have used anti-inflammatory drugs or anti- microbial drugs within a 3months period before enrollment in to the study.
3. Pregnant or lactating females.
4. Subjects who are smokers.

PROCEDURE

Periodontal parameters, i.e. Plaque index (Silness and Loe), Gingival index (Loe and Silness), Russell's periodontal index, Probing pocket depth and Clinical attachment level were assessed at baseline. Blood and saliva samples were collected from all the subjects in the morning after an overnight fast. After blood and saliva collection full mouth scaling was done. Patient was recalled after 10days from baseline for post scaling blood and saliva sample collection.

Collection of saliva samples

Unstimulated whole saliva samples were used in this study. After rinsing the mouth with plain water to remove debris, subjects were asked to spit into Sterilized saliva collection tubes. About 5ml of whole saliva was collected and stored at -80 °C and assayed biochemically for the superoxide dismutase enzyme level estimation. **(Figure 1)**

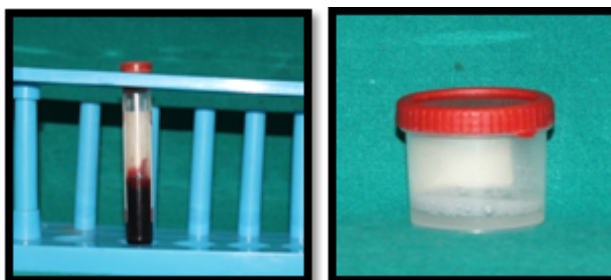


Figure 1. Serum and saliva samples.

Collection of blood samples

5ml of venous blood was drawn from the anterior cubital vein using a disposable syringe from all the subjects and transferred to sterile plain bulb. Within the twelve hours of blood collection blood was centrifuged for serum separation and the resultant serum was stored at -80 °C and assayed biochemically for the superoxide dismutase enzyme level estimation. **(Figure 2)**



Figure 2. Blood collection.

Estimation of superoxide dismutase (SOD) enzyme levels ^[4,5]

Superoxide dismutase enzyme levels were estimated in saliva and serum by using colorimetric method for measuring SOD. The Amplite Colorimetric Superoxide Dismutase Assay Kit provides a quick and sensitive colorimetric method for measuring SOD.

Statistical analysis

Statistical analysis was done by ANOVA test Descriptive statistics were expressed as mean \pm standard deviation (SD) for

each parameter at different time interval. The difference between each pair of measurement was then calculated. Data were analyzed using 't' test for paired and unpaired observations to assess changes obtained within and between groups. **(Figure 3)**

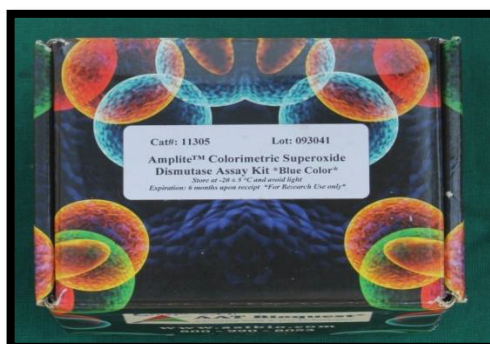


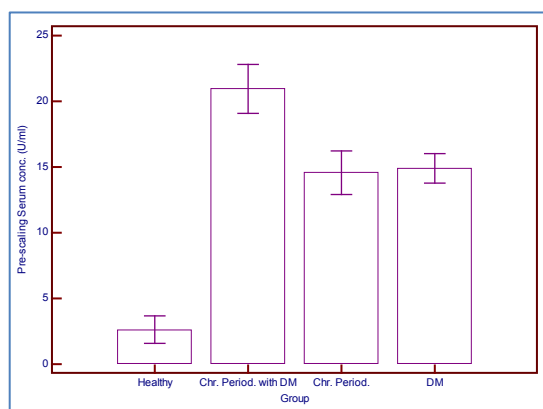
Figure 3. ELISA kit (Genxbio,pvt ltd.).

RESULTS

This study was carried out to evaluate the effect of chronic periodontitis and diabetes on the serum and salivary levels of SOD before and after SRP as described early. Serum and saliva samples were collected from all patients and analyzed biochemically for SOD activity. ELISA method was used for biochemical analysis. Results which were obtained are discussed further. **(Table 1) (Graph 1)**

Table 1. Comparison of SOD levels in serum between all the groups before SRP (Units/MI) n =No. of subjects, SD= Standard Deviation.

| GROUP | n | Mean | SD | Different from group(P<0.05) |
|-----------|----|---------|--------|--------------------------------|
| Group I | 10 | 2.6200 | 1.4559 | Group II , Group III, Group IV |
| Group II | 10 | 20.9571 | 2.0181 | Group I, Group III, Group IV |
| Group III | 10 | 14.5800 | 2.3059 | Group I, Group II |
| Group IV | 10 | 14.9000 | 1.5521 | Group I, Group II |



Graph 1. Serum SOD Levels before SRP.

Table 1 and Graph 1 shows the mean serum SOD concentration in all groups before SRP. Highest mean SOD concentration was found in Group II whereas the lowest mean SOD concentration was found in Group I.

DISCUSSION

Periodontitis is an inflammatory disease of the periodontium, which affects the supporting tissues of the teeth. The imbalance in the interactions between the pathogens and host defense results in periodontal tissue breakdown. Specific bacteria induce the release of cytokines which increase the number of defense cells and their activation. Reactive oxygen species (ROS), such as superoxide anion, are produced via the bacteria-host mediated pathway inducing tissue breakdown^[1]. Another most commonly occurring disease with inflammatory involvement is diabetes. The association between diabetes mellitus and periodontitis has long been discussed with conflicting conclusions. Both of these diseases have a relatively high incidence with a number of common pathways in their pathogenesis. Diabetes mellitus and Periodontitis are polygenic disorders with some degree of immuno-regulatory dysfunction **(Figure 4)**.

Numerous reports indicate a higher incidence of periodontitis in diabetics compared to healthy controls. The relationship between these two conditions appears bi-directional insofar that the presence of one condition tends to promote the other, and that the meticulous management of either may assist treatment of the other **(Figure 5)**.

The precise nature of inter-relationship between diabetes and chronic periodontitis is unclear. An unregulated inflammatory state has been proposed as the common mechanism underlying both conditions with an increase in cytokines, including TNF- α ,

postulated as a possible link. It was suggested that oxidative stress is a common factor in periodontal disease, type II diabetes and perhaps the 'pre-diabetic' condition and that the imbalance in redox control resulting independently from these disease states acts synergistically, and amplifies in a bidirectional manner the biochemical and clinical course of these diseases (**Figure 6**).



Figure 4. Group I case photo. Systemically and periodontally healthy patient with less than 5 mm pocket depth.



Figure 5. Group II Case Photo. Patient with chronic periodontitis and diabetes with more than 5 mm pocket depth.

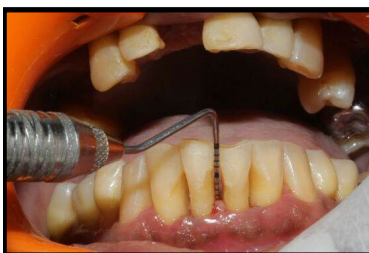


Figure 6. Group III Case Photo. Patient with chronic periodontitis with more than 5 mm pocket depth.

Recently, there has been increasing interest in reactive oxygen species and antioxidant system in the etiology of periodontitis and diabetes. It is suggested that patients with periodontal disease and diabetes are more susceptible to an imbalance of antioxidant oxidative stress situation ^[1].

Studies were conducted to evaluate the correlation between diabetes, chronic periodontitis and reactive oxygen species. Apart from these few more studies were carried out to evaluate the effect of non surgical therapy on reactive oxygen species and total antioxidant capacity in patients with chronic periodontitis (**Figure 7**).



Figure 7. Group IV case photo. Periodontally healthy patient with diabetes with less than 5 mm pocket depth.

Within mammalian tissues the most significant AO is SOD which protects the tissue against the deleterious effects of ROS, ensuring that O₂ is efficiently converted to H₂O₂ and O₂ ^[1]. SOD is released in the body as homeostatic mechanism to protect the tissues. SOD is a powerful antioxidant, produced endogenously as an intracellular enzyme ^[5] (**Table 2**).

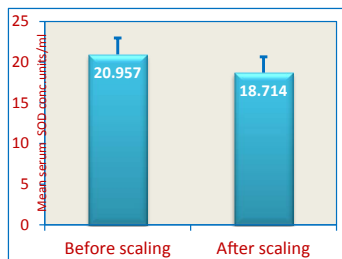
Table 2. Comparison of Serum SOD Levels (Units/MI) between Group II, Group III, and Group IV after SRP n =No. of subjects, SD= Standard Deviation.

| Group | n | Mean | SD | Different from Group (P<0.05) |
|-----------|----|--------|--------|-------------------------------|
| Group II | 10 | 18.714 | 3.087 | Group III, Group IV |
| Group III | 10 | 12.110 | 2.0867 | Group II |
| Group IV | 10 | 12.490 | 1.6636 | Group II |

Table 2 shows the mean post SRP SOD concentration.

SOD is an antioxidant enzyme that acts against superoxide, oxygen radical that is released in inflammatory pathways and

causes connective tissue breakdown and it can be detected in extra and intracellular compartments (**Graph 2**).



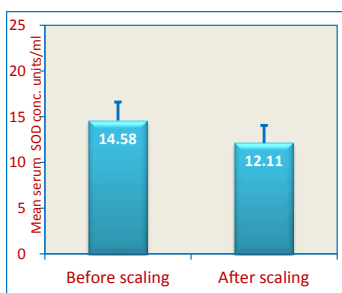
Graph 2. Serum SOD Levels in Group II Before and after SRP.

Pryor et al. in 1986 suggested that, the availability of SOD has provided a tool that allows testing physiological processes for the involvement of superoxide ion. This increase in superoxide ion may lead to increase oxidative stress, which in turn caused an increased need for SOD production to establish the ROS-AO balance to protect the tissue ^[6] (**Table 3**).

Table 3. Mean change in serum SOD levels (Units/ml).

| Change from baseline in SOD | Mean Change | % change On Day 10 | SD |
|-----------------------------|-------------|--------------------|-------|
| Group II | 2.242 | 10.698 | 0.778 |
| Group III | 2.470 | 16.941 | 0.616 |
| Group IV | 2.410 | 16.174 | 0.615 |

Joanny, Menvielle-Bourg in 2005 observed that powerful natural antioxidant enzyme (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen. Therefore, it constitutes the first and one of the main links of the defense process defending the cells against the hazardous effects of ROS. Kimura et al. suggested strong relationship between the serum concentration of extracellular (EC) SOD and the severity of both microvascular and macrovascular diabetic complications, and that serum EC-SOD concentration levels may be a marker of vascular injury ^[7] (**Graph 3**).



Graph 3. Serum SOD Levels in Group III before and SRP.

Studies have shown that periodontitis patients have compromised antioxidant defense in plasma ^[8,9], saliva ^[10], GCF ^[8] and gingival biopsy samples ^[11]. Akalin et al., ^[11] reported as SOD activity in GCF was negligent, signifying that SOD defense may be weak in GCF. Therefore, we selected serum samples for the analysis of SOD activity in our study. The idea of selecting saliva as the medium for evaluation of SOD in our study stemmed from the fact that saliva may constitute the first line of defense against free radical mediated oxidative stress in oral cavity ^[12] (**Table 4**).

Table 4. Comparison of salivary SOD levels (Units/MI) between all the groups before SRP.

| GROUP | n | Mean | SD | Different from Group (P<0.05) |
|-----------|----|--------|-------|--------------------------------|
| Group I | 10 | 1.290 | 1.042 | Group II , Group III, Group IV |
| Group II | 10 | 18.830 | 1.798 | Group I, Group III, Group IV |
| Group III | 10 | 13.240 | 1.942 | Group I, Group II |
| Group IV | 10 | 13.460 | 0.816 | Group I, Group II |

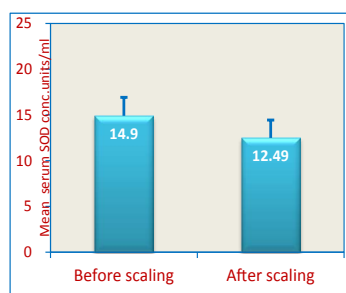
Table 4 shows the mean salivary SOD concentration in all groups before SRP. Highest mean SOD concentration was found in Group II whereas the lowest mean SOD concentration was found in Group I.

Grant et al. ^[13], Wei et al. ^[14] and Singh et al. ^[15] reported that NSPT normalized the AO levels in GCF, saliva and serum. Hence this study was conducted to evaluate the effect of scaling and root planning on periodontitis and diabetes (**Graph 4**).

Graph 2, Graph 3 and Graph 4 indicates the SOD concentration at baseline and on 10th day. Graph 5 shows the post SRP SOD concentration in **Graph 2, Graph 3, and Graph 4**. **Table 3** shows the mean change in SOD concentration from baseline to 10th day. Significant reduction in SOD concentration occurs in all the groups after SRP (**Table 5**).

Table 5 shows the mean post SRP SOD concentration. **Graph 7, Graph 8 and Graph 9** indicates the sod concentration at baseline and on 10th day. **Graph 10** shows the post SRP SOD concentration in Group II, Group III, and Group IV. **Table 6** shows

the mean change in SOD concentration from baseline to 10th day. Significant reduction in salivary SOD concentration occurs in all the groups after SRP.

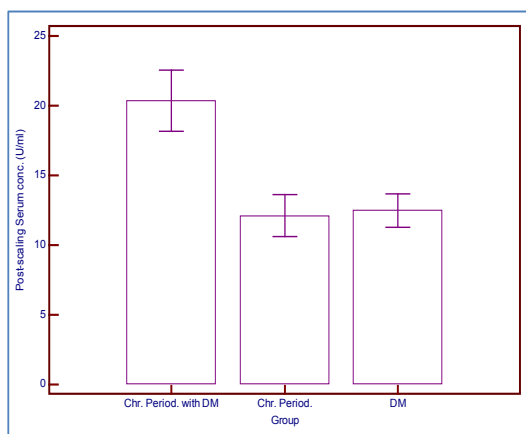


Graph 4. Serum SOD Levels in Group IV before and SRP.

Table 5. Comparison of salivary SOD levels (Units/MI) Between Group II, Group III, and Group IV after SRP n =No. of subjects, SD= Standard Deviation

| Group | n | Mean | SD | Different from Group (P<0.05) |
|-----------|----|--------|-------|-------------------------------|
| Group II | 10 | 15.970 | 1.952 | Group III, Group IV |
| Group III | 10 | 11.000 | 1.894 | Group II |
| Group IV | 10 | 10.570 | 0.984 | Group II |

In the present study, we excluded smokers, because according to Agnihotri et al. cigarette smoking is associated with significant decrease in SOD levels [4]. Garg et al. observed the delicate balance between ROS and tissue concentrations of antioxidants may be disturbed by various factors including smoking. Individuals with history of prolonged use of medications (antibiotic, anti-inflammatory therapy and antioxidant vitamins) were excluded from the study to eliminate the influence of the same on the course of periodontal disease activity, which could in turn influence the SOD activity [6] (**Graph 5**).



Graph 5. Serum SOD Levels after SRP.

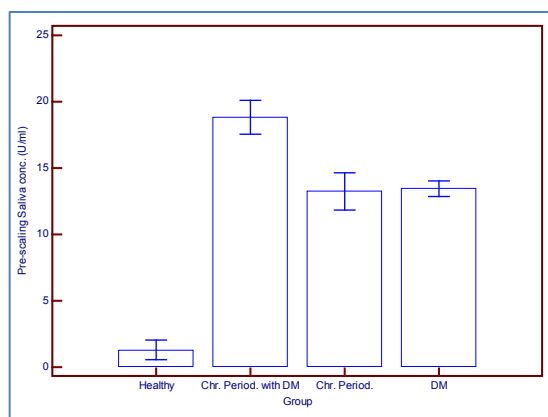
The findings of the study indicate that serum and salivary SOD concentration is higher in the periodontitis and diabetes as compare to the healthy individuals. Our results are in accordance with the various studies in literature. Aziz et al. [16], Kale et al. and Wei et al. [14], indicates the higher amount of SOD in chronic periodontitis. Sukhtankar et al. also showed the increase in SOD levels in the chronic periodontitis when compared with the healthy control group 34 In contrast to our results Trivedi et al. [17], Singh et al. [15], Baltacioglu et al. [18] and Shermin Karim et al. [19] found decreased levels of SOD in the chronic periodontitis than healthy group (**Table 6**).

Table 6. Mean change in salivary SOD levels (Units/ml).

| Change from baseline in SOD | Mean Change | % change On Day 10 | SD |
|-----------------------------|-------------|--------------------|-------|
| Group II | 2.260 | 15.180 | 1.090 |
| Group III | 2.240 | 16.910 | 0.437 |
| Group IV | 2.890 | 21.417 | 0.593 |

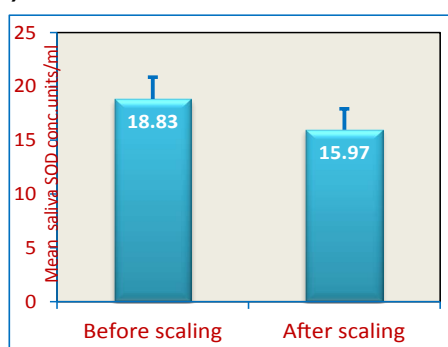
In case of oxidative stress and diabetes relation the findings of our present study linked with the literature, we found the higher levels of serum and salivary SOD levels in patients with healthy periodontium and diabetes. These results were similar to Moussa et al. [2]. Our results are in contrast with Hisalkar et al. [20] and Rahbani-Nobar et al. [21]. They found low levels of SOD in the diabetes patients when compared with the healthy control. Rahbani-Nobar et al. analyzed SOD levels in control group were decreased in diabetes than the healthy group (**Graph 6**).

Graph 6 shows the mean salivary SOD concentration in all groups before SRP. Highest mean SOD concentration was found in Group II whereas the lowest mean SOD concentration was found in Group I.



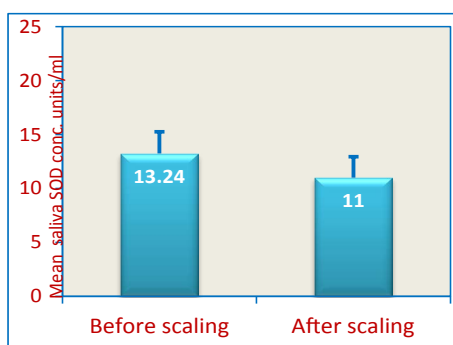
Graph 6. Salivary SOD Levels before SRP.

The various studies reported the bidirectional relationship of diabetes and periodontitis. Allen et al. in a review article reported the bidirectional relationship of the diabetes and periodontitis. Taylor et al. also showed the relation between periodontitis and diabetes. Allen et al. described link between the diabetes and periodontitis and role of oxidative stress as a mechanistic link between these both conditions ^[1,21] **(Graph 7).**



Graph 7. Salivary SOD Levels in Group II before and after SRP.

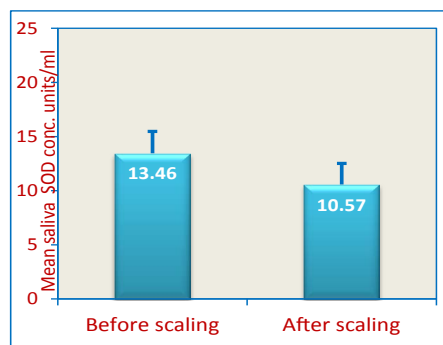
In accordance with the inter-relationship between periodontitis and type II diabetes, we found the higher levels of SOD in patients with periodontitis and diabetes than the patients with periodontitis and patients with diabetes without diabetes. It shows the additive effect of periodontitis and diabetes on SOD values **(Graph 8).**



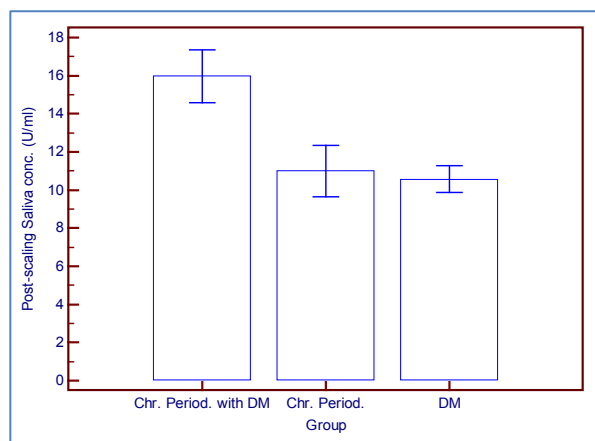
Graph 8. Salivary SOD Levels in Group III before and after SRP.

In the present study we investigated the effects of non-surgical periodontal therapy on the serum and salivary SOD levels in patients with chronic periodontitis and diabetes, patients with chronic periodontitis without diabetes and patients with healthy periodontium and diabetes, compared with systemically healthy patients. We found the significant reduction in the oxidative stress levels after SRP in all groups. These findings were same to the Sukhtankar et al., Chaudhary et al. ^[22], and Aziz et al. ^[23], Nada Novaković et al. ^[24] whereas Kim et al. ^[25] found decrease in SOD levels immediately after SRP but increased after 3months from baseline **(Graph 9).**

Karim et al. ^[19] and Yang et al. ^[25] observed the lower levels of SOD in chronic periodontitis than the healthy patients. Following SRP, superoxide dismutase concentrations significantly improved in chronic periodontitis patient group. Po-Sheng Yang et al. indicated that salivary SOD levels following SRP significantly increased in patients who had irregular dental visit patterns. After scaling, the TAOC was significantly higher in patients who had regular dental visits than in patients who had irregular dental visits. These results demonstrate the importance of scaling stimulated salivary antioxidants as prognostic biomarkers of periodontal treatment ^[26] **(Graph 10).**



Graph 9. Salivary SOD Levels in Group IV before and after SRP.



Graph 10. Salivary SOD Levels after SRP.

In the present study we found significant reduction in the oxidative stress levels after SRP in all groups. We investigated the SOD values at baseline and ten days after SRP. Long term study should be carried out to evaluate the long term effects of non-surgical periodontal therapy on serum and salivary SOD levels.

CONCLUSION

The following conclusions may be drawn from the observations in the present study:

SOD concentration was seen to be higher in periodontitis & diabetes group than healthy patients, patients with chronic periodontitis, and patients with type-II diabetes mellitus without chronic periodontitis. It shows that there is an additive effect of diabetes and chronic periodontitis on the oxidative stress.

After non-surgical therapy there was decrease in SOD concentration in patients with chronic periodontitis, patients with periodontitis and diabetes group, patients with type-II diabetes mellitus without chronic periodontitis group. This shows that there was a decrease in SOD levels with decrease in inflammatory component.

From the above results it can be concluded that non-surgical therapy is helpful to decrease the oxidative stress in inflammatory diseases like periodontitis and diabetes.

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