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COMPARATIVE ESTIMATION OF SULFIREDOXIN, TOTAL ANTIOXIDANT CAPACITY AND TOTAL PROTEIN LEVELS BEFORE AND AFTER SCALING AND ROOT PLANING IN PERIODONTITIS

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Abstract: Sulfiredoxin (SRX) is an antioxidant protein with a role in signaling through catalytic reduction of oxidative mechanisms. The present study was aimed to estimate and compare SRX in gingival crevicular fluid (GCF), total antioxidant capacity (TAOC) and total protein in saliva of healthy and periodontitis subjects before and after scaling and root planning (SRP). Periodontally healthy (n=15) and moderate to severe periodontitis subjects (n=15) were included in this study. At baseline, clinical parameters were measured and biochemical analysis were performed. After 1 month, re-evaluation of every parameter was performed in the test group. Enzyme-linked immunosorbent assay (ELISA) method was used to determine the SRX levels in GCF, TAOC and total protein estimation were performed. Paired and independent t-test were used to compare clinical and biochemical parameters. SRX was found in GCF of both healthy (1.47 ± 0.27 ng/mL) and periodontitis subjects (1.60 ± 0.43 ng/mL) and one month after SRP (1.39 ± 0.27 ng/mL). TAOC in saliva and SRX in GCF before and after SRP showed no significant difference ($p > 0.05$). The total protein levels in saliva showed significant reduction after SRP. In conclusion, the results from this study demonstrates the presence of SRX in GCF of both healthy and periodontitis subjects. In this study SRX was found to be present in GCF of healthy and periodontitis

Keywords: Periodontitis, GCF, SRX, TAOC, Total Protein

Classification number : 1.2.5. Biomolecules 2.7.2. Biomolecular materials

1. INTRODUCTION:

Periodontitis is an inflammatory condition that occurs due to the interaction of bacterial infection and an altered host defence. It leads to the breakdown of tissues that support teeth and also have an impact on overall health. Reactive oxygen species (ROS), which are overproduced in periodontitis mostly by hyperactive neutrophils, are unable to be countered by the antioxidant defence system of the body and cause damage to periodontal tissues in the process. Therefore to assess the oxidative stress related to periodontitis, total antioxidant capacity (TAOC), total oxidant status, and oxidative stress index are employed.[1-3]

One of the harmful ROS generated during the periodontal inflammation process is hydrogen peroxide, which causes tissue damage and cellular death. Catalase (CAT), Peroxiredoxin (Prx), Thioredoxin (Trx), and Glutathione peroxidase (Gpx), are examples of the enzyme antioxidants which neutralize hydrogen peroxide. The peroxiredoxin-sulfiredoxin (SRX) system of antioxidants is primarily responsible for mediating the scavenging of hydrogen peroxide.[4] This antioxidant system has attracted a lot of interest in systemic disorders.

SRX expression has been linked to a number of oxidative stress-related diseases, including atherosclerosis, chronic obstructive pulmonary disease, and a variety of carcinomas.[5] SRX and periodontal disease do not currently have a clear causal relationship.

The gold standard for early management of periodontal disease continues to be nonsurgical periodontal therapy. It may lead to a decrease in inflammation, a reduction in pocket depth, and a gain in clinical attachment.[6] Non-surgical periodontal therapy has proven to be successful in lowering plasma ROMs and improving clinical indices in patients with chronic periodontitis. [7] In patients with chronic periodontitis, scaling and root planing can also lower the overall oxidant status in the GCF.[8]

Till date, the expression of antioxidants and periodontal health remain contentious topics. While some authors suggest that the antioxidant defences elevate in patients with periodontal disease[9,10]others found conflicting evidence indicating periodontitis is connected to a lower expression of antioxidant defenses.[11,12] In previous literature, SRX levels were assessed in chronic periodontitis condition and found to be elevated in gingival tissues compared to non-periodontitis subjects in an cross sectional study.[13] But till date no literature is available regarding its assessment post periodontal therapy. In the present study SRX is assessed in gingival crevicular fluid of subjects with and without periodontitis and before and after SRP along with the estimation of protein and TAOC. It also attempts to explore the possibility of SRX being a potential biomarker in the management of periodontal disease. Sulfiredoxin, an essential antioxidant enzyme, shows potential as an indicator in periodontitis by mirroring cellular reactions to oxidative stress, critical in the disease's development and advancement. Evaluating Sulfiredoxin levels could provide valuable understandings into the well-being of periodontal tissues and their reaction to treatments like scaling and root planing.

2. MATERIALS AND METHODS:

Total 30 subjects, reporting to the Department of Periodontology, Yenepoya Dental College, systemically and periodontally healthy subjects (n = 15) were selected as control group and systemically healthy subjects with periodontitis (n = 15) were selected as test group, after receiving ethical clearance from Institutional Ethics Committee (YEC2/1086). Informed consent was obtained from all the study subjects prior to the start of the study.

The control group consisted of self reported systemically healthy subjects with clinically healthy periodontium, GI score ≤ 0.2 (absence of clinical inflammation), PPD ≤ 3 mm, and CAL = 0.

The criteria for the test group was in accordance with 2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions (Papapanou et al 2017).[14]: Subjects diagnosed with stage II and stage III with Grade A or B periodontitis, that display pocket depths ≥ 5 mm and include mostly horizontal bone loss between 15% and 33% of the root (or CAL of 3 mm to 4 mm) limited (class I) furcation involvement may also be detected.

Subjects with the following criteria were excluded: subjects with smoking habit, history of any systemic diseases that can alter the course of the periodontal disease, history of periodontal therapy in the preceding 6 months, vitamins A/C/E or any form of antioxidant therapy within the last 3 months, history of anti-inflammatory or antimicrobial therapy in past 3 months, pregnant/lactating women, history of regular use of mouthwashes in past 3 months.

GCF and saliva samples from all the study subjects were collected and stored under -80°C. Clinical periodontal examination was performed and full mouth PI (F-PI), GI (F-GI), CAL (F-CAL), PPD (F-PD) were recorded in the test group. Scaling and root planing was done and oral hygiene instructions were given to all subjects of test group. Fluoridated toothpaste and soft bristled tooth brush were prescribed to all the study subjects of test group. Subjects from test groups were recalled after one month, during which re-evaluation of clinical parameters were performed once again and GCF and saliva samples were collected and stored at -80°C for further analysis.

2.1. Methods

Saliva and GCF collection

Unstimulated whole saliva was collected into polypropylene tubes after 10 min of rinsing their mouth and transferred into the Eppendorf tubes. Samples were centrifuged to remove the cell debris and supernatants were stored at -80°C for further analysis. Pooled GCF samples from multiple sites were collected with the help of microcapillary pipettes which were inserted into the entrance of the gingival crevice/periodontal pocket after supragingival plaque removal. Collected GCF samples were transferred on whatman #1 filter paper and eluted with 50-100 µL buffer solution (phosphate buffer saline) and then were centrifuged for 15 min at 3000 rpm. After removal of filter paper the sample was

stored in a cryobox at -80°C until ELISA analysis for sulfiredoxin estimation.

Estimation of SRX levels in GCF

SRX levels in GCF was estimated using SRX (Sulfiredoxin) ELISA Kit. For SRX, the intra-assay Coefficient of Variation (CVs) was<8%, inter-assay CVs <10% and the analytic sensitivity of these assays was 0.094 ng/mL.

Salivary total antioxidant estimation

It was estimated using Koracevic et al.,(2014)[15] method. To the sample, sodium phosphate buffer, Fe-EDTA complex and H_2O_2 were added and incubated at 37°C for 1 hour. Absorbance was measured at 532 nm. In the sample, blank Fe-EDTA mixture and H_2O_2 were added after the addition of 20% acetic acid. Reagent blank contained all the similar reagents except that plasma was replaced with phosphate buffer. Standards containing 1 mmol/L uric acid was used for calibration.

Salivary total protein estimation

Total salivary protein level was estimated as per Lowry et al.,(1951)[16] method, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu+, which reacts with the Folin reagent. The reactions result in a strong blue colour, which depends partly on the tyrosine and tryptophan content. The method was sensitive down to about 0.01 mg of protein/mL, and is best used on solutions with concentrations in the range 0.01–1.0 mg/mL of protein.

Statistical analysis:

Independent sample t-test was used to compare the results between healthy patients and periodontitis. Paired t-test was used to compare the before and after treatment of periodontitis. Level of statistical significance was set at $p< 0.05$.

1. RESULTS AND DISCUSSION

Systemically and periodontally healthy subjects (n=15) were selected as control group and systemically healthy subjects with periodontitis (n=15) were selected as test group for the study. The mean age group for this study was 28.07± 7.59 years.

1.1. Measurement results obtained:

The results of the present study showed the clinical parameter's mean PI, mean GI and mean PPD and mean CAL were significantly higher in the test group than the control group. (Table 1) and following one month after SRP, all the clinical parameters showed statistically significant reduction from the baseline. (Table 2)

Table 1 . Intergroup comparison of clinical parameters before SRP (Scaling and Root planing) (n=15+15)

Baseline	Control	Test group before SRP	Test Statistics	*p* Value
Plaque Index	1.16 ± 0.35	2.47 ± 0.32	-10.52	<0.0001***
Gingival Index	0.75 ± 0.36	2.45 ± 0.39	-12.20	<0.0001***
Probing pocket depth (in mm)	2.87 ± 0.36	6.05 ± 0.56	-18.14	<0.0001***
Clinical attachment level (in mm)	1.75 ± 0.69	6.07 ± 0.85	-15.17	<0.0001***

Data represented in mean ± SD. Statistical test used: Paired t test. Level of significance: $p<0.05$ was considered significant. $p<0.0001$(***) was considered very highly significant. Clinical parameters such as Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL) were measured for both control and test group before SRP. Probing pocket depth and Clinical attachment level were expressed in mm.

Table 2 . Intragroup comparison of clinical parameters before and after SRP among subjects with periodontitis (n=15)

Test group clinical parameters	Before SRP	One month after SRP	Test Statistics	*p* value

Plaque Index	2.47 ± 0.32	1.46 ± 0.36	9.95	<0.0001***
Gingival Index	2.45 ± 0.39	1.18 ± 0.41	10.23	<0.0001***
Probing Pocket Depth (in mm)	6.05 ± 0.56	4.43 ± 0.78	14.54	<0.0001***
Clinical Attachment Level (in mm)	6.07 ± 0.85	4.88 ± 0.66	10.38	<0.0001***

Data represented in mean ± SD. Statistical test used: Paired t test. Level of significance: $p<0.05$ was considered significant. $p<0.0001$(***) was considered very highly significant. Clinical parameters such as PI, GI, PPD, CAL were measured for test group before and after SRP. Probing pocket depth and Clinical attachment level were expressed in mm.

At baseline, present study found mean SRX level in GCF in control group was 1.47 ± 0.27 ng/mL and test group showed slightly higher baseline mean SRX levels at 1.60 ± 0.43 ng/mL, which was not statistically significant (p value = 0.33). This suggests that, at baseline, there is no significant variation in SRX levels in the GCF between control and test groups (Table 3). The test group showed slightly lower SRX levels following SRP at 1.39 ± 0.27 ng/mL. There was no statistically significant difference among the test group before and one month after SRP with a p value of 0.11. (Table 4)

Table 3. Intergroup comparison of biochemical parameters of control and test groups at baseline

Baseline	Control	Test	Test statistics	*p* value
Srx levels in GCF (ng/mL)	1.47 ± 0.27	1.60 ± 0.43	-0.98	0.33
Total protein levels in saliva (µg/mL)	1097.82 ± 551.58	2110.13 ± 1028.51	-3.35	<0.05**
Total antioxidant capacity in saliva (µmol/L)	17.66 ± 7.22	24.43 ± 14.39	-1.62	0.11

Data represented in mean ± SD. Statistical test used: Independent sample t test. Level of significance: $p<0.05$ was considered significant. Srx levels in GCF are expressed in ng/mL, Total protein levels in saliva are expressed in µg/mL, TAOC in saliva is expressed in µmol/L.

Table 4 Intragroup comparison of biochemical parameters of test groups before and after SRP

Baseline	Test group (before SRP)	Test group (One month after SRP)	Test Statistics	*p* value

SRX levels in GCF (ng/mL)	1.60 ± 0.43	1.39 ± 0.27	1.67	0.11
Total protein levels in saliva (µg/mL)	2110.13 ± 1028.51	565.79 ± 249.99	6.15	<0.0001
Total antioxidant capacity in saliva (µmol/L)	24.43 ± 14.39	19.38 ± 5.11	2.65	0.18

Data represented in mean ± SD. Statistical test used: Paired t test. Level of significance: $p<0.05$ was considered significant. Biochemical parameters were measured for test group prior to SRP and one month post SRP. SRX levels in GCF are expressed in ng/mL, Total protein levels in saliva are expressed in µg/mL, TAOC in saliva is expressed in µmo/L.

At baseline, total protein levels in saliva for the test group (2110.13 ± 1028.51 µg/mL) was significantly higher than the control group (1097.82 ± 551.58 µg/mL) with a *p* value <0.0001 (Table 3). The total protein levels in saliva for the test group before SRP (2110.13 ± 1028.51 µg/mL) exhibited significantly lower total protein levels (565.79 ± 249.99 µg/mL) following SRP with *p* value <0.0001. (Table 4)

At baseline, TAOC in saliva for the control group (17.66 ± 7.22 µmol/L) showed no significant difference with that of the test group (24.43 ± 14.39 µmol/L) with a *p* value = 0.11 (Table 3). The TAOC in saliva for the test group before SRP (24.43 ± 14.39 µmol/L) reduced one month after SRP (19.38 ± 5.11), however, the difference was not statistically significant with *p* value of 0.18 (Table 4).

1.2. Discussion on characteristics

Antioxidants are the substances that counteract ROS and support the delicate equilibrium between oxidants and antioxidants in the body. In periodontitis, the prolonged inflammatory response and elevated ROS generation can potentially overwhelm the antioxidant defence system. Consequently, therapies targeted at increasing antioxidant capacity or decreasing excessive ROS generation will have therapeutic value in the treatment of periodontitis.[17]SRX is an enzyme that specifically resolves hyperoxidized versions of these antioxidant proteins, hence modifying the activity of PRX and contributing to the regulation of cellular redox equilibrium.[18]

The present study aimed to estimate and compare SRX levels in GCF, and total antioxidant capacity (TAOC) and total protein in the saliva of healthy and periodontitis subjects before and after SRP. Clinical parameters were recorded and compared among both the control groups and test groups (Table 1). At baseline, the clinical parameters (PI, GI, mean PPD, mean CAL) of control group were significantly lower as compared to that of the test group. The clinical parameters were compared before

SRP and one month after SRP in the test group (Table 2), which showed a significant reduction after SRP. This indeed emphasises the role of SRP in controlling the inflammation in periodontitis.

A definitive and scientifically persuasive proof that GCF is a source of several biomarkers suggest the presence of a disease with specifics of active and passive sites was reported by Barros et al., (2016).[19] A thorough review of the host defence mediators and their expression in the GCF surrounding teeth was given by Kaur et al., (2017),[20] and these findings specifically supported the use of GCF as a universal diagnostic tool. The present study results prove the presence of SRX in GCF of both healthy (1.47 ± 0.27 ng/mL) and in periodontitis subjects (1.60 ± 0.43 ng/mL) at baseline. The SRX levels in GCF of periodontitis subjects were slightly higher than that of healthy subjects but the difference was not statistically significant. This result was in contradiction to that of results achieved by Ramesh et al., (2018),[13] which showed significantly higher SRX levels in the gingival tissue of periodontitis group compared to that of healthy subjects. A study by Kennet et al., (1995),[21] found Tryptase activity in gingival mast cells and is found in gingival tissue in higher concentrations than in GCF during inflammatory conditions. Similarly, GCF might have comparatively lesser amounts of SRX than that present in the gingival tissues. In addition, the present study observed the SRX levels in the GCF of test group before SRP (1.60 ± 0.43 ng/mL) decreased one month after SRP (1.39 ± 0.27 ng/mL), the difference was not significant (p = 0.11). This inconclusive difference could be due to the shorter recall interval of one month after SRP for the estimation of SRX levels. Studies have shown that, in periodontitis subjects tissue response to SRP takes longer duration of more than one month to reflect in biological tissue samples.[22,23]

At baseline, the salivary total protein levels was significantly higher (p < 0.001) in the test group (2110.13 ± 1028.51 µg/mL) as compared to the control group (1097.82 ± 551.58 µg/mL**).** These elevation of total protein levels in saliva can include both host and the bacterial proteins. Results of the present study are in alignment with a study by Kejriwalet al., (2014).[24] The total protein levels of saliva in test group before SRP (2110.13 ± 1028.51 g/ml) reduced significantly one month after SRP (565.79 ± 249.99 µg/mL), indicating the effectiveness of SRP. This result wes in accordance with studies by Kim et al., (2010),[25] Mohammad et al., (2018),[26] Sharma et al., (2015)[27]. This highlights tracking alterations in salivary protein levels could serve as a valuable method to determine the response following periodontal treatment.

The results of present study did not show significant differences in the TAOC of saliva between the control (17.66 ± 7.22 µmol/L) and test groups (24.43 ± 14.39 µmol/L) at baseline. And although TAOC levels reduced one month after SRP (19.38 ± 5.11 µmol/L) when in comparison with test group at baseline, the difference was not significant. These results were in concordance with a study by Nisha et al., (2022),[22] and Toczewska et al., (2020).[28] Contrary to the results of the present study, Novakovic et al., (2014),[29] found a significant increase in TAOC level after SRP and Aziz et al., (2013),[23] found

that SRP significantly reduces the TAOC. The variability of TAOC observed in saliva is due to the contribution of various intrinsic and extrinsic factors in the oral environment such as diet, smoking, alcohol consumption, and exposure to environmental pollutants, individual variations in antioxidant defence mechanisms, genetic predisposition, age, systemic health status, and medications.[30]

The present study was a novel attempt to identify the presence of SRX in GCF of both healthy and periodontitis patients before and after scaling and root planing, and estimating the clinical parameters, TAOC and total protein levels in saliva. Based on the findings obtained from the present study, SRX was present in GCF both in healthy and periodontitis conditions, but in a significantly lower amount. Periodontal treatment reduced the SRX levels however, not at statistically significant levels. Since the present study did not show a significant difference in SRX level before and after treatment, SRX may not be a potential diagnostic or biological marker in periodontitis patients for evaluating the efficacy of scaling and root planing.

It would be appropriate to consider three months recall after SRP for the evaluation of biological markers in GCF or saliva. In the present study, salivary TAOC estimation was done. Future investigations may focus on estimating TAOC in GCF of periodontitis subjects as it is most localized with distinctive characteristics of the site, leading to appropriate diagnosis and treatment planning.

CONCLUSION:

The present study was a unique attempt in estimating the salivary total protein and total antioxidant capacity and comparing withthe SRX levels in GCF before and after periodontal treatment. The observation and interpretation from this study clearly demonstrates the presence of SRX in GCF of both healthy and periodontitis subjects. Thus, estimation of SRX levels can be performed with a much simpler way for future studies. The findings suggest there was no significant changes in the levels of SRX in GCF and TAOC in saliva between healthy and periodontitis subjects, and also before and one month after SRP in periodontitis subjects. On the other hand, total protein levels in saliva showed significant reduction after SRP.

Future studies may be conducted with a larger sample size, multiple recall visits and longer follow-up period of at least three months for evaluating the role of SRX in periodontitis patients and to monitor its levels following periodontal treatment and site-specific collection of GCF to evaluate TAOC. Further, correlation of SRX levels with TAOC and total protein levels, could provide insights into the relative importance of different antioxidants in combating oxidative stress, association with disease activity, treatment outcomes and help tailor therapeutic strategies.

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Credit authorship contribution statement. Author 1: Methodology, Investigation, Funding acquisition. Author 2: Formal analysis. Author 3: Formal analysis, Supervision. Author 4: Formal analysis. Author 5:Biochemical analysis. Author 6 : Biochemical analysis. Author 7 : Formal analysis, Supervision

Declaration of competing interest. The authors declare we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval : The study titled "Comparative estimation of Sufiredoxin, Total antioxidant capacity and Total protein levels before and after scaling and root planing in periodontitis" with protocol number YEC2/1086 was approved by Yenepoya Ethics Committee 2 after reviewed through an expedited review process.

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