Evaluation of glutathione level in gingival crevicular fluid in periodontal health, in chronic periodontitis and after nonsurgical periodontal therapy: A clinicobiochemical study

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Abstract

Context: Periodontitis is predominantly due to exaggerated host response to pathogenic microorganisms and their products which causes an imbalance between the reactive oxygen species-antioxidant in gingival crevicular fluid (GCF). Glutathione is an important redox regulator in GCF and maintenance of stable reduced glutathione (GSH):oxidized glutathione (GSSG) ratio is essential for periodontal health. **Aims:** The present study was undertaken to evaluate and compare the level of glutathione and redox balance (GSH: GSSG ratio) in GCF of chronic periodontitis patients, periodontally healthy controls and also to evaluate the effect of nonsurgical periodontal therapy on the level of glutathione and redox balance during 3 months postoperative visit. **Study Design:** Baseline GCF samples were collected from 20 chronic periodontitis patients and 20 periodontally healthy subjects for GSH and GSSG levels estimation. Periodontitis patients were recalled 3 months postnonsurgical periodontal therapy to re-sample GCF. **Materials and Methods:** GSH and GSSG levels were measured by high-performance liquid chromatography. The values were statistically analyzed by Paired *t*-test. **Results:** The mean GSH and GSSG values in GCF were found to be significantly lower in periodontitis patients pre- and 3 months post-nonsurgical periodontal therapy, compared with those in the control group subjects. In addition, the successful nonsurgical therapy even though leading to a significant improvement in the GSH and GSSG levels, does not restore glutathione concentration to the levels seen in healthy subjects. **Conclusion:** Successful nonsurgical periodontal therapy leads to significant improvement in the redox balance (GSH: GSSG ratio) in chronic periodontitis patients.

Keywords: Chronic periodontitis, gingival crevicular fluid, glutathione

Introduction

Periodontitis is an inflammatory disease process which is initiated mainly by plaque biofilm.[1] The primary etiologic agents for gingival and periodontal diseases are predominantly Gram-negative anaerobic or facultative anaerobic bacteria within the biofilm. However, the majority of periodontal tissue destruction is mediated by an exaggerated host response to these pathogenic microorganisms and their products.[2]

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The predominant inflammatory cells within the connective tissues and epithelium of the gingiva are the polymorphonuclear leukocytes; therefore, the release of their lysosomal enzymes and the generation of extracellular reactive oxygen species (ROS) are thought to be a major factor in the etiology of local tissue damage. In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant (AO) defense capacity and when this equilibrium shifts in favor of ROS, the imbalance has been implicated as one of the progressive and pathogenic factors for periodontal disease.[1]

Glutathione is reported to be one of the most important redox regulators, which controls inflammatory process. In its reduced form, glutathione (GSH) is an important AO (radical scavenger). The preliminary studies on GSH content of gingival crevicular fluid (GCF) suggested that fluid levels were lower in periodontitis patients than controls.[3]

The aim of the present case–control study was to evaluate and compare the level of glutathione and redox balance (GSH: Oxidized glutathione [GSSG] ratio) in GCF of chronic periodontitis patients, periodontally healthy controls and also to evaluate the effect of nonsurgical periodontal therapy on the level of glutathione and redox balance during 3 months postoperative visit.

Materials and Methods

Study was conducted on forty patients in the age group of 30–50 years, who reported to the Department of Periodontics, Dayananda Sagar College of Dental Sciences, Bangalore. Ethical clearance was taken from the Institutional Ethical Committee before the commencement of the study. Verbal and written informed consent was obtained from all the participants. The subjects were divided into three groups: Group A consisted of 20 periodontally healthy controls (no bleeding on probing, probing pocket depth [PPD] ≤3 mm, no clinical attachment loss [CAL]); Group B consisted of 20 patients with chronic periodontitis (presence of bleeding on probing, at least two nonadjacent sites per quadrant with PPD ≥5 mm and CAL ≤3 mm); Group C consisted of 20 chronic periodontitis patients whose samples were re-collected 3 months after the completion of nonsurgical periodontal therapy.

The subjects with the history of smoking habit, usage of vitamin supplements, usage of anti-inflammatory or antibiotic medication in past 3 months, any form of systemic diseases, pregnant, menopause or lactating women, regular usage of mouthwash were excluded from the study.

After enrolment, all volunteers were appointed for collection of GCF samples, which were taken 1-day after recording clinical measures (bleeding on probing, PPD and CAL) to prevent contamination of GCF with blood associated with the probing of inflamed sites. GCF samples were collected from all the groups from the mesiobuccal and distolingual sites on each of three teeth (molar, premolar, and canine or incisor) in upper left or in upper right quadrants, providing six samples by placing the micropipette at the entrance of the gingival sulcus and gently touching the gingival margin. No attempt was made to specifically select sites with deep pockets because samples were pooled per subject to ensure sufficient assay sensitivity and the patient was used as the unit of analysis. Site-specific differences were therefore not analyzed. Sites were isolated using cotton rolls and gently air dried prior to sampling. In all the chronic periodontitis patients (Group B), conventional nonsurgical periodontal therapy in the form of scaling and root planing (SRP) was performed under local anesthesia on a quadrant-by-quadrant basis.

Patients were recalled 3 months posttherapy to repeat clinical measures and to re-sample GCF (Group C). A 3 months recall was chosen to allow for initial healing and to reduce the risk of re-infection/disease re-activation. GCF samples from six sites per individuals were pooled and eluted into 1 mM Ethylenediaminetetraacetic acid (EDTA), 5 mg/L cresol red, 0.2 M boric acid in 3.5% perchloric acid stabilizing medium (300 μl) to prevent oxidation of labile AO species. GSH and GSSG were measured by high-performance liquid chromatography using a fluorimetric detector after derivatization with dansyl chloride. Concentrations of GSH and GSSG were determined by reference to standard curves acquired from a parallel measurement of external standards and adjustment for variations in derivatization and sample delivery to the column using an internal standard (10 mM g-Glu-Glu). The data gathered from the study were subjected to appropriate statistical analysis.

Statistical analysis

The mean values (mean ± standard deviation) were reported for each parameter. The mean GSH value and GSSG value for the Group A was compared to that of the Group B using Student's unpaired *t*-test. The significant level $P \leq 0.05$ was considered statistically significant.

The change in mean values of GSH and GSSG within the Group B and Group C were statistically evaluated using Student's paired *t*-test. In addition to that the change in the mean GSH: GSSG ratio from Group B to Group C was also evaluated using Student's paired *t*-test.

Results and Observations

In all the groups, the mean GSH and GSSG values were detected in the millimolar (mM) range. The mean value for GSH in the GCF from Group A (control group) was found to be 1456.01 ± 84.63 mM. The mean value for GSH in the GCF from Group B and Group C was found to be 738.31 ± 111.81 mM and 1295.42 ± 116.03 mM, respectively [Table 1].

The mean GSH value was found to be higher in Group A compared to that in Group B as well as in Group C and the difference between them was found to be statistically significant ($P < 0.001$) [Figure 1 and Table 2].

The mean value for GSSG in the GCF from Group A was found to be 1287.56 ± 105.69 mM. The mean value for GSSG in the GCF from Group B and Group C was found to be 610.58 ± 105.69 m and 760.81 ± 86.20 mM, respectively [Table 3].

The mean GSSG value was found to be higher in Group A compared to that in Group B and Group C. Furthermore, the difference between them was found to be statistically significant ($P < 0.001$) [Figure 2 and Table 4].

The change in mean GSH value and GSSG value from Group B to Group C was found to be statistically significant ($P < 0.001$) [Table 5 and 6].

The change in mean GSH: GSSG ratio was evaluated using paired *t*-test. The statistical evaluation has shown that the GSH: GSSG ratio in the Group B had increased from 1.23 to 1.73 after 3 months posttreatment (Group C) and it was found to be statistically significant ($P < 0.001$) [Table 7 and Figure 3].

Table 1: GSH values for control group (Group A), Group B, and Group C

GSH	Mean	SD	SEM
Group A	1456.01	84.63	18.92
Group B	738.31	111.81	25.00
Group C	1295.42	116.03	25.94

GSH: Reduced glutathione, SD: Standard deviation, SEM: Standard error of mean

Table 2: Comparison of the GSH levels between control group and study groups

GSH	Mean difference from the control group (Group A)	*t*	*P*
Group B	717.700	22.889	<0.001*
Group C	160.594	5.001	<0.001*

**P*<0.001 is statistically significant. GSH: Reduced glutathione

Table 3: GSSG values for Group A, Group B and Group C

GSSG	Mean	SD	SEM
Group A	1287.56	105.69	23.63
Group B	610.58	99.46	22.24
Group C	760.81	86.20	19.27

GSSH: Oxidized glutathione, SD: Standard deviation, SEM: Standard error of mean

Table 4: Comparison of the level of GSSG between control group and study groups

GSSG	Mean difference from the Group A	*t*	*P*
Group B	676.98	20.86	<0.001*
Group C	526.75	17.27	<0.001*

**P*<0.001 is statistically significant. GSSH: Oxidized glutathione

Table 5: Comparison of GSH levels within the study groups

GSH	Mean	SD	SEM	Mean difference	*t*	*P*
Group B	738.31	111.81	25.00	−557.106	−14.880	<0.001*
Group C	1295.42	116.03	25.94			

**P*<0.001 is statistically significant. GSH: Reduced glutathione, SD: Standard deviation, SEM: Standard error of mean

Table 6: Comparison of GSSG levels within the study groups

GSSG	Mean	SD	SEM	Mean difference	*t*	*P*
Group B	610.58	99.46	22.24	−150.235	−8.016	<0.001*
Group C	760.81	86.20	19.27			

**P*<0.001 is statistically significant. GSSH: Oxidized glutathione, SD: Standard deviation, SEM: Standard error of mean

Discussion

In the present study, the mean GSH and GSSG values were found to be significantly lower in the GCF from chronic periodontitis patients pre- and 3 months post-nonsurgical periodontal therapy, compared with those detected in GCF from control group subjects. In addition, we have also showed that successful nonsurgical therapy even though leading to a significant improvement in the GSH and GSSG levels, does not appear to restore glutathione concentration to the levels seen in health but raised the redox balance (GSH: GSSG ratio) in GCF. These results are in broad agreement with the published reports on GCF and salivary levels of ROS and antioxidant molecules in periodontitis patients.[3,4]

Our study findings are consistent with the previous studies carried out by Carlsson *et al*. 1993 and Chu *et al*. 2002, which

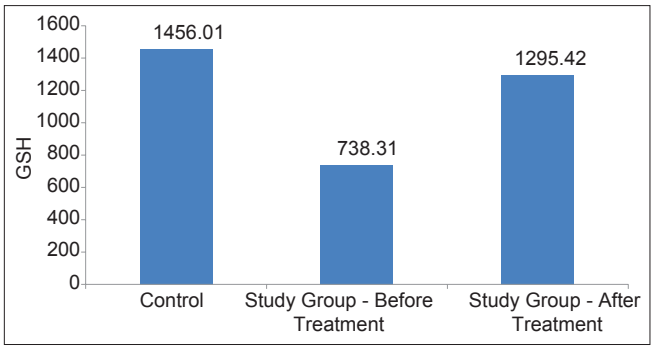

Figure 1: Comparison of mean reduced glutathione value between control group (Group A) and study groups (Group B and Group C)

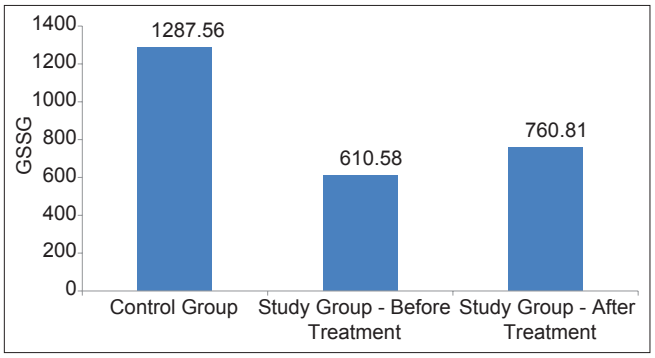

Figure 2: Comparison of mean oxidized glutathione value between control group and study groups

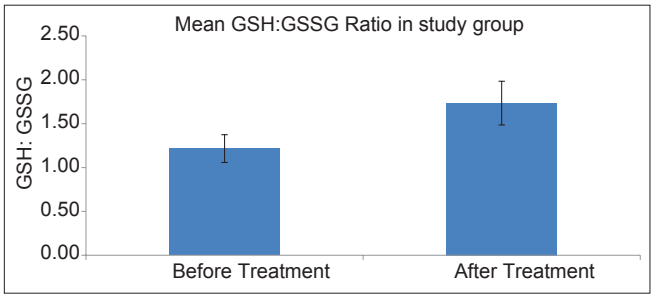

Figure 3: Comparison of reduced glutathione:oxidized glutathione ratio within study groups

Table 7: Comparison of GSH: GSSG ratio within study groups

GSH: GSSG	Mean	SD	SEM	Mean difference	*t*	*P*
Group B	1.22	0.16	0.04	−0.518	−10.631	<0.001*
Group C	1.73	0.25	0.06			

**P*<0.001 is statistically significant. GSH: Reduced glutathione, GSSH: Oxidized glutathione, SD: Standard deviation, SEM: Standard error of mean

showed that despite the capacity of certain periodontal bacteria to metabolize GSH, biofilm removal via successful nonsurgical periodontal therapy does not fully restore glutathione concentrations within GCF, implying a negligible impact of the microbial environment upon *in vivo* GCF glutathione levels.[5,6]

Although the source of the high concentrations of GSH and GSSG in GCF remains to be elucidated, it is likely that neutrophils are the major contributors. The main mechanism behind the reduced concentration of GSH level in diseased state may be because of an inborn defect in the γ-glutamyl pathway of GSH synthesis or the presence of certain putative pathogens which readily degrade GSH into hydrogen sulfide.[7]

A recent comprehensive review concluded that oxidative stress is at the heart of the periodontal tissue damage that results from the host–microbial interactions.[4] Overproduction of cytokines, proteinases, and reactive species contributes to the chronic nature of the inflammatory lesion. There is still debate as to whether AO depletion is a cause of disease or a consequence of the tissue damage that accompanies disease progression.[8]

The method used for GCF collection and storage may influence the anti-oxidant capacity upon analysis. Hence, in this present study, we have pooled and eluted the GCF into 1 mM EDTA, 5 mg/L cresol red and 0.2 M boric acid in 3.5% perchloric acid stabilizing medium (300 μl) to prevent oxidation of labile AO species.

Clearly, utilizing the patient as the unit for analysis in our study is one of the accepted approaches, which prevents site-based variability that may confound the resulting data. This approach of collecting GCF only from those selected sites may be one limitation of our study because that may lead to underestimation of the oxidative stress since oxidative stress may be greater at the sites with deeper pocket where the inflammatory burden is greater.

GSH and GSSG concentrations in GCF remain lower than control patients, implying a reduced buffering capacity against ROS activity in periodontitis patients, even following successful therapy. This may constitute a deficiency in innate immunity in periodontitis patients, rendering them more susceptible to oxidative stress and its sequel, but further research is needed to confirm such a thesis. If demonstrated, then such findings open up the potential to develop novel therapeutic approaches based upon elevating the GSH buffering capacity within tissues using pharmacological interventions, such as the use of the GSH-promoting drug N-acetyl-cysteine, an approach currently under investigation in the management of rheumatoid arthritis. Alternatively, given that GSH is the principal chain-breaking AO in the extracellular environment, micro-nutritional approaches to boost tissue AO concentrations, may preserve GSH and create an anti-inflammatory tissue redox state. Currently, such approaches are being actively pursued in the preventive management of various chronic inflammatory diseases including periodontitis where oxidative stress underpins a key role in the pathogenesis.[3,9]

One such future prospect of utilizing AOs for treatment of gingivitis and periodontitis currently under study includes the Coenzyme Q_{10} topical gel which contains ubiquine.[10,11] Another randomized controlled clinical trial has reported promising results with Co-Q_{10} (Hydro-Q-Sorb) in solitary use as well as when it was used as an adjunct to SRP for the treatment of plaque-induced gingivitis.[12]

Recently, a number of mouth washes and tooth powders are getting promoted by the clinicians, which have a dynamic combination of AOs, that is, propolis, Coenzyme Q_{10}, Green tea catechins, and *Aloe vera* with an antibacterial and anti-inflammatory role.[13] Thus, in summary, the identification of many key AOs opened a new avenue to futuristic concepts in periodontal therapy.

Conclusion

The present study has demonstrated that GSH and GSSG concentrations in the GCF of periodontally disease patients remain lower than that of healthy subjects. Therefore "AOs" may be recommended as a strong adjunctive therapy, and this will, in addition, have a great impact because people tend to abide increasingly to preventive protocol programs rather than curative regimens. Research is still underway regarding this aspect and emphasis is upon the longitudinal studies and their results.

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