ORIGINAL ARTICLE

Relationship between gingival inflammation and total glutathione

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Abstract

Aim: Glutathione, which is known to be the main antioxidant, is the foremost redox regulator in the control of inflammatory processes. The goal of this study was to investigate the levels of total glutathione, which plays a central role in cellular antioxidant defense, in the gingival crevicular fluid (GCF) and plasma of individuals with gingivitis and periodontally healthy.
Materials and Methods: Fifteen periodontally healthy subjects and 15 individuals with gingivitis were enrolled in the study. Samples of GCF, plasma, and clinical measurements were attained before and one month after non-surgical periodontal therapy. GCF and plasma levels of total glutathione were analyzed using spectrophotometric assay.
Results: It was found that the levels of total glutathione in GCF were lower in gingivitis group than those in the periodontally healthy group (P<0.05), while there was no statistical difference in the plasma levels of total glutathione between gingivitis and periodontally healthy groups (P>0.05). Also, a statistically significant rise in GCF and plasma total glutathione levels in gingivitis group was found after periodontal therapy compared to baseline (P<0.05). Furthermore, there was a negative correlation between total glutathione and gingival index (P<0.05).
Conclusion: Findings of this study showed that GCF and plasma levels of total glutathione could be influenced by gingival inflammation. In addition, it was revealed that the importance of periodontal therapy in the patients with gingivitis.

Keywords: Gingivitis; Glutathione; Gingival Crevicular Fluid; Plasma.

INTRODUCTION

Periodontal diseases are infectious diseases that related to the interaction between periodontopathogens and host immunoinflammatory responses. Neutrophil enzymes and reactive oxygen species (ROS) release during this interaction (1). ROS are derived from normal metabolic reactions, however, higher concentrations of ROS may result in tissue damage via the lipid peroxidation, protein denaturation and DNA damage (2). A review has shown that increasing ROS and/or decreasing anti-oxidative condition act a crucial role in the advancement of periodontal disease (3).

Glutathione is a tripeptide found in all cells which play a central role in cellular antioxidant defense and has important roles in the neutralization of harmful compounds (4). It is stated that glutathione is a useful indicator for the antioxidant protection mechanism in oxidative stress-induced chronic inflammatory diseases (5,6).

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It is stated that glutathione is a useful indicator for evaluating the antioxidant protection mechanism in oxidative stress-induced chronic inflammatory diseases (5, 6).

Previous studies reported that lower gingival crevicular fluid (GCF) and plasma glutathione levels in patients with chronic periodontitis compared with periodontally healthy individuals were increased after initial periodontal therapy (4,7-10). It was suggested that there was an adverse relation between glutathione levels and the severity of periodontal disease (10).

Gingivitis is the most common inflammatory periodontal disease worldwide affecting 80% of the population (11). If gingivitis is untreated, it may result in alveolar bone destruction and connective tissue attachment loss (12). As yet, there is a study evaluating total glutathione levels in individuals with gingivitis. Buduneli et al. examined total glutathione levels in saliva in individuals with gingivitis and periodontally healthy individuals (13). They found no significant difference in the salivary total glutathione levels between the both groups (13). Thus, authors indicated that the measurement of antioxidant capacity in DOS could be more valuable for periodontal inflammation compared to saliva (13).

Thus, we hypothesized that gingival inflammation may decrease total glutathione levels in the GCF and plasma

in individuals with gingivitis and periodontal therapy could have affirmative effects on these levels. Thus, the aim of this study was to evaluate the clinical periodontal parameters and analysis of total glutathione levels in GCF and plasma in the individuals with gingivitis before and after non-surgical periodontal therapy.

MATERIALS and METHODS

Study population
Thirty subjects, 16 males, and 14 females, in the age range of 25-53 years, received to the Periodontology Department of Bulent Ecevit University, Faculty of Dentistry, either for dental therapy or only for the dental check up from March 2013 to January 2014 were recorded in the study. All subjects gave their written informed consent and the protocol of the study was approved by the Ethics Committee of the Faculty of Medicine, Bülent Ecevit University, Zonguldak, Turkey in accordance with the Helsinki Declaration of 1975, as revised in 2002 (Protocol ID: 2013-25-12/02).

The diagnosis was made according to the criteria defined by the 1999 International World Workshop for the Classification of Periodontal Diseases and Conditions (14). Gingivitis group was designed as having pocket probing depth (PPD) ≤ 3mm, that were positive for bleeding on probing (BOP) in at least 2 different quadrants and gingival index (GI) score >1. Periodontally healthy group was designed as healthy if the full-mouth PPD was ≤3mm, GI = 0 (lack of clinical inflammation). In both groups, there were no signs of clinical attachment loss and radiographic evidence of alveolar bone loss (i.e., the distance between the cemento-enamel junction [CEJ] and bone crest < 3 mm at >95% of the proximal tooth sites). The inclusion criteria for all subjects in the study were possessed of at least 20 teeth excluding third molars, and teeth with advanced decay. Fifteen individuals with clinically healthy periodontium (8 males and 7 females, with an age range of 25-33 years) and 15 individuals with gingivitis (8 males and 7 females, with an age range of 25-53 years) were recruited.

Inclusion criteria were never-smokers, no history of systemic disease, no patients had been under periodontal therapy and medicine for at least 6 months before the study, no pregnancy or lactation and no alcohol or antioxidant vitamin consumption.

Clinical Measurements
Patient periodontal status was determined by measuring PPD, GI (15), plaque index (PI) (16) and BOP (17). The level of periodontal bone loss was determined by taken full-mouth periapical radiographs. All clinical parameters were measured at six sites per tooth using (mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual, and midlingual) a William's periodontal probe (Hu-Friedy, Chicago, IL.) calibrated in millimeters by the same examiner (FÖD).

Collection of Samples
All samples were collected in the morning following an overnight fast, during which patients were requested not to drink (except water) or eat. Before sample collection, the individuals were checked for protocol adherence.

In order to avoid irritation, GCF samples were collected 2 days after the clinical measurements, in the morning between 8:00 and 10:00. GCF samples were collected from a mesio-buccal and disto-palatal site on each tooth (molars, premolars, canines/incisors). In gingivitis group, GCF samples were obtained from teeth with BOP and without CAL. In the healthy group, GCF samples were collected from teeth exhibiting PD<3 mm without CAL and BOP. Six GCF samples were collected from each patient. The area was isolated with cotton rolls, saliva contamination elimination was ensured, and it was slightly air dried. GCF was sampled with paper strips (Periopaper; Ora Flow Inc., Amityville, NY, USA). Paper strips were placed into the crevice until mild resistance was felt (intracrevicular method) and left in the position for 30 seconds (18). Strips contaminated with blood or saliva were discarded. A modification of the protocol described by Curtis et al. (19) was used for GCF elution from the periopapers. Each sampled strip was placed in a disposable tube and stored at –40°C until analyzed.

Five milliliters of venous blood was taken from the antecubital vein by using a standard venipuncture method. The plasma was separated from blood by centrifugation at 1000 x g for 10 min. The plasma samples were stored at -40°C until analyzed.

Biochemical Analysis
GCF and plasma samples were used to determine total glutathione levels with Glutathione Assay Kit (Cayman Chemical Company, Item No. 703002 AnnArbor, MI, USA). The absorbance was measured at 260 nm in a spectrophotometer. The data were expressed as µM/L.

Periodontal Treatment
All patients were motivated and instructed for daily plaque control. Non-surgical periodontal therapy was applied to individuals with gingivitis. Periodontal therapy was applied at distinct periods appropriate to their periodontal condition with the mean frequency visits for 3 weeks. Therapy included an intensive hygiene phase, full-mouth scaling and root planning, and maintenance and monitoring of oral hygiene. Clinical data and samples were obtained before and after one month non-surgical periodontal therapy.

Statistical Analyses
Statistical analysis was performed using a commercially available software program (SPSS 19.0; SPSS Inc., Chicago, IL). The Shapiro-Wilk test was used to investigate whether or not the data were normally distributed. Continuous variables with unequal variances were compared by means of Welch and Tamhane's T2 post-hoc test for BMI, PD, and total glutathione. The comparison of the age, GI, PI and BOP was analyzed using the Kruskal-Wallis non-parametric test followed by post hoc group comparisons with the Bonferroni-adjusted Mann–Whitney U test. Paired Student's t-test or Wilcoxon rank-sum test was used to comparing the measurements at two points (baseline and after treatment). The Spearman's rank correlation test was also used to detect the relationship between biochemical and clinical findings. P<0.05 was considered to be statistically significant.

RESULTS

Demographic values and mean values of clinical measurements are showed in Table 1. The full-mouth BOP, PI, and GI values were statistically higher in the gingivitis group than periodontally healthy group (P<0.05), while there was no significant difference in the PPD values between both groups (P>0.05). The mean PI, GI, and BOP values were statistically lower in the gingivitis group after non-surgical periodontal therapy (P<0.05). Regarding age and proportion of genders, there was no significant difference between both groups (P>0.05).

The levels of GCF and plasma total glutathione are shown in Table 2. Plasma total glutathione levels in gingivitis group was lower than the periodontally healthy group, however, no significant differences were found total glutathione levels in plasma between gingivitis and periodontally healthy groups (P>0.05). On the other hand, plasma total glutathione levels in gingivitis patients increased after non-surgical periodontal therapy compared to baseline (P<0.05).

The levels of GCF total glutathione were significantly lower in patients with gingivitis than those in periodontally healthy individuals at baseline (P<0.05). A statistical increase in GCF total glutathione levels in gingivitis group was found after therapy compared to baseline (P<0.05). Also, a statistically significant negatively correlation was found between total glutathione with PI (r=-0.584, P=0.000), GI (r=-0.829, P=0.000) and BOP (r=-0.791, P=0.000) when all groups were examined together (P<0.05).

Table 1. Demographic values of the Study Groups

		Gingivitis	Periodontal healthy
n		15	15
Male:female		8:7	8:7
Age		31.53	29.60
PI	Baseline	1.66±0.32*	0.20±0.19
	Post-therapy	0.42±0.22**	-
GI	Baseline	1.83±0.32*	0.00±0.00
	Post-therapy	0.41±0.15**	-
BOP (%)	Baseline	65.39±18.51*	0.00±0.00
	Post-therapy	10.18±5.50**	-
PPD (mm)	Baseline	1.69±0.25	1.48±0.14
	Post-therapy	1.69±0.25	-

Data are expressed as the mean ± standard deviation.
* Statistically significant difference from periodontal healthy group (P<0.05)
** Statistically significant difference from baseline (P<0.05)

Table 2. The levels of total glutathione at baseline and after therapy in GCF and plasma (µM/L)

	Plasma			GCF		
	Baseline	Post-therapy	*P value*	Baseline	Post-therapy	*P value*
Gingivitis	40.31±4.98	43.89±2.93**	<0.01	43.71±7.18*	51.01±7.75**	0.000
Periodontal healthy	46.97±10.04	-		56.73±7.98	-	

Data are expressed as the mean ± standard deviation.
* Statistically significant difference from periodontal healthy group (*P*<0.001)
** Statistically significant difference from baseline (*P*<0.01)

DISCUSSION

Gingivitis is a periodontal disease that set off with a pathogenic biofilm on the teeth sustained by a host immune-inflammatory reaction (20). It was indicated that the imbalances between ROS and antioxidant levels may act a critical role in the onset and progression of many inflammatory oral diseases (21). Changes in the levels of ROS and antioxidant during gingival inflammation may affect immuno-inflammatory response to plaque bacteria of the host. Evaluating of the local and systemic antioxidant levels in the gingival inflammation may be beneficial in determining the severity of the disease. To our knowledge, this is the first study to determine levels of plasma and GCF total glutathione in individuals with gingivitis. Also, our study evaluated correlation relationship between clinical parameters and total glutathione levels.

In this study, GCF was collected to analyze the total glutathione levels. The biochemical analysis of GCF may be beneficial in the determination of tissue destruction in periodontium and may provide exact data including the pathogenesis of periodontal disease (22). A collection of GCF is a non-invasive method and is usually preferred 30 s time of sampling by using paper strips

(23). Lamster et al. emphasized that the total amount of an ingredient in the GCF in place of its concentration is more suitable when examining the relationship between the GCF ingredient and periodontal diseases (24). Thus, in this study, levels of total glutathione in GCF is evaluated mainly on its total amount.

The aim in the treatment of gingivitis is to prevent plaque accumulation and to eliminate periodontal tissue inflammation (12). In our study, periodontal therapy in patients with gingivitis led to improvement in clinical periodontal parameters. In accordance with our study, Buduneli et al. have shown that PI and BOP values significantly increased in individuals with gingivitis compared with periodontally healthy individuals and then these values significantly decreased after periodontal therapy in individuals with gingivitis (13).

Lomaestro and Malone stated that glutathione ensures an efficient antioxidant prevention against oxidative damage of the free radicals (25). Di Renzo et al. proposed that reduced glutathione levels are related to chronic inflammatory diseases (6). Also Chapple et al. indicated that the antioxidant content of GCF was locally produced from three sources including plaque bacteria, neutrophils and sulcus epithelium (4). Chu et al. stated that the levels of glutathione in the GCF may decrease due to consuming glutathione of many periodontopathogens bacteria (26). Moreover, Tsai et al. stated that improvement clinical parameters after initial periodontal treatment may influence increasing of antioxidant defense (9). Thus, Grant et al. detected that GCF levels of total glutathione in patients with periodontitis were lower than in periodontally healthy individuals and then these levels were augmented after periodontal therapy in patients with periodontitis, however, this increase was not statistically significant (8). The findings of the present study showed significantly lower levels of GCF total glutathione in the gingivitis group compared with the periodontally healthy group and these levels increased after therapy compared to the baseline. We also found negative correlations among GCF levels of total glutathione and GI, PI, BOP in all groups. Based on these findings, the present authors suggested that gingival inflammation may impact on the GCF levels of total glutathione and antioxidant defense may increase as a result of the improvement of gingival inflammation.

Panjamurthy et al. exhibited that levels of glutathione in the plasma were lower in periodontitis patients compared with the healthy subjects (7). On the other hand, Biju et al. found that levels of glutathione in the serum were lower in periodontitis group compared with the control group (10). Thus, the authors noted that there may be a relationship between the severity of periodontal disease and glutathione levels (10). Contrary to these studies, our study demonstrated no difference in the levels of plasma glutathione between gingivitis and periodontally healthy groups. The discrepancy may be attributed that plasma samples were obtained from gingivitis patients in this study, but from periodontitis patients in other studies. Periodontitis which characterized by connective tissue attachment loss and

alveolar bone resorption is a more severe form of periodontal disease (12). Our data also demonstrate that the plasma levels of total glutathione in gingivitis group increased after treatment compared to baseline. This increase can be explained by the systemically improvement in the total glutathione levels after treatment because of a reduction in the gingival inflammation. In view of our data, the GCF and plasma levels of total glutathione can be useful in the assessment of the pathophysiological process associated with periodontal therapy in patients with gingivitis.

CONCLUSION

The findings of the present study showed that gingival inflammation may be an important effect on GCF and plasma total glutathione levels in patients with gingivitis. The findings suggest that the measurement of total glutathione levels in the GCF may be useful to determine the severity of the gingival inflammation and the efficacy of periodontal therapy. In addition, consumption of foods containing antioxidants may be recommended additionally to periodontal therapy to decrease the impact of gingival inflammation on glutathione levels in patients with gingivitis. The determination of this relationship may provide new ideas for the treatment of gingivitis. Studies involving larger group sizes and long-term are required to clarify the relationship between antioxidant defense and gingival inflammation.

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