PROTEIN CARBONYL LEVELS IN SERUM, SALIVA AND GINGIVAL CREVICULAR FLUID IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTALITIS

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Abstract Objective: This study aims at evaluating the degree of protein carbonyl (PC) levels in serum, gingival crevicular fluid (GCF) and saliva in patients who suffer from chronic periodontitis (CP) and generalized aggressive periodontitis (GAP).

Materials and methods: A total of 110 individuals took part in the study. Of this number, 35 were CP patients, 43 GAP patients, and the remaining 32 were healthy controls. Measurements regarding the serum, saliva and GCF PC levels were obtained by high-performance liquid chromatography.

Results: No statistically significant difference was found in serum PC levels between the groups (P > 0.05). In terms of salivary levels, the CP group demonstrated a significantly higher level (P < 0.05) of PC level compared to the GAP group. However, the difference was not found statistically significant when the comparison was drawn with the control group (p > 0.05).

The GCF PC level in the CP group had a significantly higher level of concentration compared to the other groups (P < 0.05), whereas the relevant values in the control group were higher than the values in the GAP group (P < 0.05). GCF PC total values (/30 s) were higher in the CP group than the remaining groups (P < 0.05), whereas the relevant values in the GAP group were higher than the values in the control group (P < 0.05). It could be stated that GCF PC levels were significantly correlated, either positively or negatively, with all clinical periodontal parameters (p < 0.05).

Conclusions: The results obtained suggest that PC levels of serum and salivary in periodontitis, when compared to periodontal health, do not seem to change considerably. However, in the CP group, a statistically significant increase in PC levels of GCF was observed. This finding suggests the salient role of local protein carbonylation in the periodontal area in CP. That the CP group

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1. Introduction

Periodontitis is described as a chronic inflammatory disease which is characterized by the destruction of periodontal connective tissue and alveolar bone. This disease is very common in society, and it was found to have a negative effect on the systemic health and life quality of people (Cullinan and Seymour, 2013). Several studies indicate that a considerable amount of the soft and hard tissue injuries are due to the host immunoinflammatory response (Baltacioglu et al., 2014b; Chapple and Matthews, 2007; Page and Kornman, 1997; Pihlstrom et al., 2005). In recent years, there have been a number of studies related to host-related factors responsible for periodontal destruction (Bartold and Van Dyke, 2013). Reactive oxygen species (ROS) are among the destructive host factors which are associated with the pathogenesis of periodontal diseases (Baltacioglu et al., 2014b; Bartold and Van Dyke, 2013; Chapple and Matthews, 2007).

ROS are known to be strong oxidants (Agarwal et al., 2005) and may form via a large number of physiologic and nonphysiologic processes (Baltacioglu et al., 2014b; Chapple, 1997; Halliwell, 2000; Waddington et al., 2000). According to Chapple and Matthews (2007), oxygen-derived free radicals and non-radical derivatives of oxygen are included in these processes. While a small amount of ROS are inevitable in some biochemical processes, such as intracellular messaging, cellular differentiation, growth arrest, apoptosis, and immunity (Roberts and Sindhu, 2009), ROS could prove to be detrimental for several biologic molecules, containing lipids, proteins, and DNA (Akalin et al., 2007; Baltacioglu et al., 2014a; D’Aiuto et al., 2010; Waddington et al., 2000) unless they are neutralized by antioxidant systems. A significant threat to be noted here is oxidative stress. It is described as a process where the dynamic redox balance between oxidants and antioxidants is intensely shifted toward oxidative potentials (Akalin et al., 2007; Baltacioglu et al., 2008; Chapple, 1997). In the light of the documented literature, the crucial role of oxidative stress is apparent in >100 disorders as well as periodontal diseases (Akalin et al., 2007; Baltacioglu et al., 2008, 2014b; Chapple, 1997).

Oxidative stress can be determined through the analysis of ROS reaction products in biomolecules (Chapple and Matthews, 2007). Indirect biomarkers of exercise-induced oxidative stress, such as protein oxidation, lipid peroxidation (LPO), total antioxidant capacity (TAOC) and total oxidant status (TOS) are routinely measured to give an indication of altered redox balance (Akalin et al., 2007; Baltacioglu et al., 2014a, 2014b; Chapple and Matthews, 2007). So far, the examination of the carbonyl groups in proteins has received considerable attention because of its stability and ease in detection (Baltacioglu et al., 2008; Cai and Yan, 2013; Wadley et al., 2016). Generally, protein carbonylation is an irreversible non-enzymatic process resulting from ROS and downstream products of oxidative processes (Baltacioglu et al., 2008; Dalle-Donne et al., 2003, 2006; Wadley et al., 2016). Protein carbonyl (PC) groups are either directly or indirectly derived. In direct derivation, amino acid side chains are directly oxidized, or indirect derivation occurs through conjugation by reactive species such as advanced lipoxidation end products and advanced glycation end products (Baltacioglu et al., 2008; Dalle-Donne et al., 2003, 2006). Previous studies in relation to the formation of PC levels fail to distinguish between direct protein oxidation and the formation by the previously added oxidized molecules (Baltacioglu et al., 2008; Dalle-Donne et al., 2003, 2006; Stadtman and Levine, 2003).

Currently, two chief forms of periodontitis are recognized: chronic (CP) and aggressive periodontitis (AP). Compared to chronic periodontitis, generalized aggressive periodontitis (GAP) leads to early tooth loss because of episodic and rapid destruction of periodontal tissues (Armitage, 1999). Even though immunological differences can be evidenced, histopathological features or profiles of microbial colonization cannot be a reference point to distinguish chronic and aggressive periodontitis lesions (Armitage, 1999; Kulkarni and Kinane, 2014; Ryder, 2010). Moreover, there is dearth of research targeting the difference in oxidative stress between the pathologic mechanisms of these two diseases (Baltacioglu et al., 2014a, 2014b; D’Aiuto et al., 2010; Konopka et al., 2007).

Oxidative stress was found to be connected with the periodontitis severity and bone destruction (Baltacioglu et al., 2014). However, it is not yet clear how protein oxidation affects GAP and plays role in the differences in the pathologic mechanism of both periodontal diseases. To address this gap, this study aims to compare the effects of systemic and local protein oxidation on chronic and aggressive forms of periodontitis and to determine the role of protein oxidation in the severity of periodontal disease. Therefore, this study examines PC levels in serum, saliva, and gingival crevicular fluid (GCF) in chronic and aggressive periodontitis subjects. The data obtained were compared with those from healthy controls, and the relationship between PC levels and clinical periodontal status was investigated.

2. Material and methods

2.1. Clinical studies

2.1.1. Study groups

A total of 110 individuals, comprising 35 patients with CP (CP Group; 18 males and 17 females, aged 23 to 42 years), 43 patients with GAP (GAP Group; 22 males and 21 females, aged 22 to 37 years), and 32 periodontally healthy controls (Control Group; 17 males and 15 females, aged 26 to 38 years) were involved in the study. Purposive sampling was used to identify the patients from those who visited Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, either for routine controls or due to periodontal-related
problems. Individuals admitted to our clinic for routine periodontal follow-up, and individuals without any periodontal problems were the control group participants.

The participants were clinically and radiographically evaluated for CP and GAP according to the criteria accepted by the American Academy of Periodontology in 1999 (Armitage, 1999; Page and Eke, 2007). The clinical periodontal criteria for the diagnosis of the groups were as follows: the CP group had <30% periodontal bone loss with teeth having a clinical attachment level (CAL) ≥ 5 mm and periodontal probing depth (PD) ≥ 5 mm in one or more sites of the teeth in multiple sites of all four quadrants of the mouth. Patients were included in the GAP group if they were aged 18 to 40 years, had ≥20 teeth, CAL ≥ 6 mm, and PD ≥ 6 mm at two or more sites in ≥12 teeth during screening, with at least three teeth involved other than the first molars and incisors. The diagnosis of GAP required familial aggregation, with at least one other family member presenting with or having a history of periodontal disease (Armitage, 1999; Page and Eke, 2007). All patients were observed to have poor oral hygiene. The amount of accumulated plaque was reported to match with the amount of clinical attachment loss (AL), and there was no restoration in the teeth. The control group were comprised of periodontally healthy individuals, and they had no recorded history of periodontal problems, with PD ≤ 3 mm, and AL ≤ 1 mm, good oral hygiene, no gingival inflammation, and no tooth restorations.

The participants, all of whom were never-smokers, were reported to have no previous periodontal treatment, and history of systemic disease. Moreover, non-use of antibiotics, anti-inflammatory or any other drugs in the last 3 months, and non-consumption of alcohol or antioxidant vitamin tablets were reported. All individuals are inhabitants of Black Sea coastal region in northeast Turkey. Thus, the nutrition habits of the participants were similar. To be specific, fish, vegetables, and fruit, which are rich in antioxidants and the most common products in the region as a result of the natural conditions of the area were the main nutritional sources.

The information about the study was initially provided for the participants, whose written consent was taken. The research study protocol was signed and approved between the Karadeniz Technical University Faculty of Medicine and Ethics Committee.

2.1.2. Clinical measurements

For all individuals, PD and CAL (measured using a periodontal probe), 1 gingival index (GI) (Loe and Silness, 1963), gingival bleeding index (GBI) (Muhlemann and Son, 1971), and plaque index (PI) (Silness and Loe, 1964) were recorded. In an attempt to identify the level of periodontal bone loss, periapical full-mouth radiographs of the participants were obtained. The PD and CAL were measured in duplicate at six tooth sites (mesial, median, and distal points at the buccal and palatal aspects), while the GI, GBI, and PI were measured in duplicate at four sites (mid-buccal, mesial, mid-palatal, and distal) of all teeth. These clinical and radiographic evaluation were carried out by the same examiner (EB) from the Department of Periodontology. The participants were reported to have ≥20 teeth in total.

2.1.3. Collection of samples

The individuals were asked to refrain from eating or drinking in the morning. As a further step to increase the reliability and validity of the study, the participants were also checked for protocol adherence prior to sample collection. The samples obtained were collected two days after the clinical measurements. As for the saliva samples, whole unstimulated ones were collected. These samples were obtained following the procedures described by Broek et al. (2004). In line with those procedures, the participants were told not to swallow any saliva, thereby enabling the measurement of salivary flow rates (FRs). The salivary FR was measured by dividing the volume by collection time (Akalin et al., 2007; Baltacioglu et al., 2014b; Broek et al., 2004). Before conducting the analysis, the saliva was centrifuged for 10 min. at 4000g at 4 °C. Then the supernatant fraction was aliquoted and stored in liquid nitrogen until analysis (Akalin et al., 2007; Baltacioglu et al., 2014b; Sculley and Langley-Evans, 2003).

The CP and GAP groups which had ≤5 mm PD and 30% bone loss were chosen for the GCF samples. Each patient gave ten GCF samples which were collected from mesio-buccal and disto-palatal sites on each of five teeth (maxillary pre-molar and incisor teeth). Cotton rolls were used to isolate the area, and they were also employed to eliminate saliva contamination. The samples were collected in 30 s with standardized paper strips following the instructions described by Rudin et al. (1970). GCF volume was measured using a precalibrated electronic device. The same procedure was followed to gather GCF samples from the control group. Ten Periopaper strips were placed in glass tubes and they were treated with 500 ml of PBS (pH 6.5). After a 30-min. elution at room temperature, the Periopaper strips were removed and stored in liquid nitrogen until analysis (Broek et al., 2004; Chapple et al., 1999).

Venous blood samples were collected in plain tubes. The tubes were kept at 4 °C for a further 30 min. prior to centrifugation at 1500g for 10 min. The Serum samples were aliquoted into cryogenic vials and stored in liquid nitrogen. Each of the patients was used as the unit of analysis.

2.2. Laboratory studies

2.2.1. Determination of protein carbonyl (PC) content

Protein carbonyl content of serum, saliva and GCF samples were measured by the method of Griffiths et al. (2017) by high-performance liquid chromatography. The samples were derivatized with 20 mM 2,4-dinitrophenyl hydrazine (DNPH). 100 µL derivatized samples were treated with 100 µL 12% sodium dodecyl sulfate (SDS). 200 µL DNPH were added in SDS-treated samples and gently vortexed for 10 min at 25 room temperature. After that incubation 50 µL sample was applied to column and that was eluted with at a flow rate of 2 ml sodium phosphate buffer/min. Eluent were monitored at 276 nm and 360 nm.

The PC concentrations in serum, saliva and GCF were expressed as mol carbonyl/mol protein. The GCF PC levels were calculated in the total GCF amount, and this amount

1 Hu-friedy, chicago, IL.

2 Periopaper, oraflow, amityville, NY.

3 Periotron 8000, oraflow.

4 HPLC, Agilent 1100 series HPLC systems; Agilent, Waldbronn, Germany; column, ZORBAX GF 450, 92 × 250 mm and 92 × 100 mm guard column.
2.3. Statistical analyses

The Shapiro–Wilk test was used to measure the normality of the distribution of the data. The difference between the CP, GAP and control group variables that have normal distribution (PD, CAL, serum and salivary PC, GCF PC concentration, GCF PC/30 s., age, GCF volume, saliva FR) was analyzed by one-way ANOVA and Tukey’s tests. Normally distributed data (GI, GBI, and PI), however, was analyzed using Kruskal–Wallis test and the Mann–Whitney U-tests employing the Bonferroni correction. The correlations between clinical parameters and serum, saliva and GCF PC levels were determined by the Pearson’s correlation coefficient. A p < 0.05 value was considered statistically significant. After performing a post hoc power calculation, a power of >0.8 at the p < 0.05 level for serum, saliva and GCF PC outcomes was obtained. All statistical analyses were performed with a software program.5

3. Results

3.1. Clinical findings

The mean values of the clinical parameters are listed in Table 1. All clinical parameters were higher in the periodontitis groups than in the control group and in the GAP group compared with the CP group (P < 0.05) (Table 1).

No significant difference was observed in sex among the groups (P > 0.05). The mean age in the CP group was higher than that in the GAP group (P < 0.05), whereas no statistically significant difference was found between the control group and the CP and GAP groups (P > 0.05). The mean saliva FR was 0.37 ± 0.09 ml/min. in the CP group, 0.35 ± 0.09 in the GAP group and 0.40 ± 0.08 ml/min. in the control group. The saliva FR in the control group was higher than that in the GAP group (P < 0.05), whereas no difference was noted between the CP group and the CP and GAP groups (P > 0.05).

The mean ± SD values of GCF volumes are shown in Table 2. The mean GCF volume of the GAP group were higher than those of the CP and control groups (P < 0.05), and in the CP group compared with the control group (P < 0.05).

3.2. Laboratory findings

A comparison of serum PC levels between the groups showed no significant differences (P > 0.05). The mean ± SD of salivary PC levels in the groups were as follows: in the CP group 2.27 ± 0.47 mol carbonyl/mol protein, in the GAP group 1.84 ± 0.54 mol carbonyl/mol protein and in the control group 2.05 ± 0.46 mol carbonyl/mol protein. It was found that the CP group had significantly higher salivary PC levels compared to the GAP one (P < 0.05). However, the difference was not found to be significant when the comparison is drawn between the CP and control group (P > 0.05) (Table 2, Fig. 1).

The mean GCF PC concentration in the CP group was calculated to be 11.55 ± 2.58 mol carbonyl/mol protein, 7.92 ± 1.78 mol carbonyl/mol protein in the GAP group and 9.79 ± 1.67 mol carbonyl/mol protein in the controls. GCF PC concentration values in the CP group were higher than the values in the GAP and control groups (P < 0.05), whereas the relevant values in the control group were higher than the ones in the GAP group (P < 0.05). PC concentration levels were the highest in the CP group and the lowest in the GAP group (Table 2, Fig. 1).

The mean GCF PC total (30 s) was measured as 47.09 ± 10.31 mol carbonyl/mol protein in the CP group, 39.47 ± 9.65 mol carbonyl/mol protein in the GAP group and 25.25 ± 7.44 mol carbonyl/mol protein in the controls. GCF PC total values (30 s) in the CP group were higher than in the GAP and control groups (P < 0.05), whereas the relevant values in the GAP group were higher than the values in the control group (P < 0.05). PC total values were the highest in the CP group and the lowest in the control group (Table 2, Fig. 1).

Regarding the GCF PC values, statistically significant positive and negative weak correlations were found with all clinical periodontal parameters (p < 0.05) (Table 3), while there was not a significant correlation between the serum and salivary PC levels (p > 0.05). A moderate (relatively strong) positive correlation was observed between all clinical parameters and GCF PC total values, while there was a weak negative correlation between GCF PC concentration values and all clinical parameters. At the same time, PC concentrations in saliva and GCF samples exhibited a weak positive correlations with GCF.

4. Discussion

Although findings on how oxidative stress could be influential in periodontal diseases have been explored in various oxidative stress parameters (Baltacioglu et al., 2014a, 2014b; Chapelle and Matthews, 2007; Tongue et al., 2011), there are few studies examining protein carbonyl levels (Allen et al., 2011; Baltacioglu et al., 2008; Pradeep et al., 2013; Sculley and Langley-Evans, 2003). On the other hand, the way in which oxidative stress is followed by clinical and molecular differences in chronic and aggressive periodontitis is now a current research topic (Baltacioglu et al., 2014a, 2014b; D’Aiuto et al., 2010; Konopka et al., 2007). Therefore, additional findings on oxidative stress in CP and GAP could give new dimensions to why periodontal infections are reported to have a chronic or aggressive course. This study is the first study examining GAP protein carbonylation and comparing it systemically and locally in both periodontal disease groups.

Protein carbonylation causes oxidative damage, and such damage may result in the deterioration of the protein function (Baraiibar et al., 2013; Dalle-Donne et al., 2003, 2006). Recently, these carbonyls have served as biomarkers of oxidative stress due to their advantage of stability and early formation (Baltacioglu et al., 2008; Baraiibar et al., 2013; Dalle-Donne et al., 2003, 2006). Moreover, previous studies point to the connection between protein carbonylation and oxidative stress and pathological conditions (Baraiibar et al., 2013; Dalle-Donne et al., 2003, 2006). Indeed, the observation of an increase in the systemic and local PC levels of the individuals

5 SPSS v.16.0 for Windows, IBM, Chicago, IL.
with chronic periodontitis and obesity, diabetes mellitus and acute coronary syndrome compared to the ones with periodontal health, suggests that oxidative stress plays a role in the relationships of these systemic diseases/conditions with periodontal disease (Allen et al., 2011; Dalle-Donne et al., 2003, 2006; Pradeep et al., 2013). Besides these findings, in another PC study, systemic PC levels were found to be significantly higher in the CP group than the control group (Baltacioglu et al., 2008). Furthermore, after the examination of saliva of the subjects with periodontitis, Sculley & Langley-Evans (2003) observed significantly higher PC levels and lower TAOC compared to the control group.

Unlike the above findings, serum PC levels in this study did not change in both periodontitis groups compared to the control group.

### Table 1 Comparison of clinical periodontal parameters among the groups.

| Parameter (mm) | Group n | CP: 35 | GAP: 43 | Control: 32 | \( \bar{X} \pm SD \) or median (min–max) | Test statistics | P  |
|---------------|--------|--------|--------|-------------|-------------------------------------|----------------|----|
| PD CP         | 3.61 ± 0.30 | F = 790.775 | 0.001\(^{A}\) | | | |
| GAP*          | 4.91 ± 0.55 | | | | | |
| Control       | 0.89 ± 0.36 | | | | | |
| CAL CP        | 3.92 ± 0.24 | F = 713.415 | 0.001\(^{A}\) | | | |
| GAP*          | 5.52 ± 0.67 | | | | | |
| Control       | 1.14 ± 0.42 | | | | | |
| GI CP         | 1.40 (1–2) | \( \chi^2 = 86.406 \) | 0.001\(^{B}\) | | | |
| GAP*          | 2.18 (1–3) | | | | | |
| Control       | 0 (0–0) | | | | | |
| GBI CP        | 2 (1–3) | \( \chi^2 = 81.695 \) | 0.001\(^{B}\) | | | |
| GAP*          | 2.6 (2–3) | | | | | |
| Control       | 0 (0–0) | | | | | |
| PI CP         | 1 (0–2.5) | \( \chi^2 = 76.918 \) | 0.001\(^{B}\) | | | |
| GAP*          | 2 (0.9–3) | | | | | |
| Control       | 0 (0–0.3) | | | | | |

PD, probing depth; CAL, clinical attachment level; GI, gingival index; GBI, gingival bleeding index; PI, plaque index; GAP, generalized aggressive periodontitis; CP, chronic periodontitis.

\(^{A}\) One-way ANOVA and Tukey tests.

\(^{B}\) Kruskal Wallis, Mann Whitney U test with Bonferroni correction.

\(*\) The difference is significant compared to the Control group (p < 0.05).

\(\dagger\) The difference is significant compared to the GAP group (p < 0.05).

### Table 2 Comparison of PC levels in serum, saliva and GCF among the groups.

| Parameter                      | Group n | CP: 35 | GAP: 43 | Control: 32 | \( \bar{X} \pm SD \) or median (min–max) | Test statistics | P  |
|-------------------------------|--------|--------|--------|-------------|-------------------------------------|----------------|----|
| Serum PC conc. (mol carbonyl/mol protein) | CP     | 6.51 ± 1.29 | F = 0.574 | 0.001\(^{A}\) | | |
|                              | GAP    | 6.36 ± 0.50 | | | | |
|                              | Control| 6.61 ± 1.12 | | | | |
| Saliva PC conc. (mol carbonyl/mol protein) | CP\(\dagger\) | 2.27 ± 0.47 | F = 7.250 | 0.001\(^{A}\) | | |
|                              | GAP\(\dagger\) | 1.84 ± 0.54 | | | | |
|                              | Control | 2.05 ± 0.46 | | | | |
| GCF PC conc. (mol carbonyl/mol protein) | CP\(\dagger\) | 11.55 ± 2.58 | F = 34.311 | 0.001\(^{A}\) | | |
|                              | GAP\(\dagger\) | 7.92 ± 1.78 | | | | |
|                              | Control | 9.79 ± 1.67 | | | | |
| GCF PC /30 s (mol carbonyl/mol protein) | CP\(\dagger\) | 47.09 ± 10.31 | F = 47.377 | 0.001\(^{A}\) | | |
|                              | GAP\(\dagger\) | 39.47 ± 9.65 | | | | |
|                              | Control | 25.25 ± 7.44 | | | | |
| GCF Volume                   | CP\(\dagger\) | 4.08 ± 0.36 | F = 273.062 | 0.001\(^{A}\) | | |
|                              | GAP\(\dagger\) | 4.98 ± 0.49 | | | | |
|                              | Control | 2.62 ± 0.42 | | | | |

PC, Protein carbonyl; GAP, generalized aggressive periodontitis; CP, chronic periodontitis; GCF, gingival crevicular fluid; conc, concentration; 30 s, 30 s.

\(^{A}\) One-way ANOVA and Tukey tests.

\(\dagger\) The difference is significant compared to the Control group (p < 0.05).

\(\dagger\) The difference is significant compared to the GAP group (p < 0.05).
periodontal health. Moreover, although PC values in saliva were significantly higher in the CP group, saliva PC values in both periodontitis groups did not change compared to periodontal health. There are various studies in the literature showing that various oxidative stress parameters outside of PC levels were increased/decreased/not changed in periodontitis patients compared to periodontal health. There are various studies in the literature showing that various oxidative stress parameters outside of PC levels were increased/decreased/not changed in periodontitis patients compared to periodontal health (Akalin et al., 2007; Baltacioglu et al., 2014a, 2014b; Chapple and Matthews, 2007). For example, in a limited number of studies investigating LPO levels in periodontal disease and health, it was shown that systemic and local malondialdehyde (MDA) increases or does not change in periodontitis (Akalin et al., 2007; Baltacioglu et al., 2014b). Oxidative stress in chronic and aggressive periodontitis has been reported to have a relationship with the increasing TOS and decreasing TAOC rather than LPO, and also appeared no alterations in systemic LPO (Baltacioglu et al., 2014b). Furthermore, in previous studies that used 8-hydroxy-2-deoxyguanosine levels and diacron-oxygen-reactive metabolites as markers of oxidative stress, no significant difference between AP and CP was noted (D’Aiuto et al., 2010; Konopka et al., 2007). On evaluating the results of the present study and those of previous studies together, the lack of difference in levels in serum and saliva between periodontitis and periodontal health and the higher PC values in CP than in AP indicate that TAOC, TOS, and other oxidative stress parameters, rather than protein oxidation, may be more effective in periodontitis. In fact, in the previous our study, an increased TOS concentration in patients with GAP was detected, suggesting that the severe breakdown occurred as a result of neutrophil hyperactivity (Baltacioglu et al., 2014a).

GCF PC total and concentration findings in the CP group were significantly higher compared to periodontal health, similar to the findings in the literature (Allen et al., 2011; Atabay et al., 2017; Baltacioglu et al., 2008; Pradeep et al., 2013). However, GCF PC concentrations were observed lower in the GAP group compared to the CP group and periodontal health. As the increase in GCF volume due to inflammation in the GCF group dilutes the PC levels, the GCF PC concentration values in the GAP group may have been decreased than in the other groups (Baltacioglu et al., 2008). Also, in support of this idea, total GCF values in the GAP group were found to be higher compared to the values in controls. It is worth noting that, in the event of local production, remarkable increase in GCF flow will dilute the CGF concentration. Thus, the total amount is recommended for consideration (Layik et al., 2000). In this study, positive correlations between GCF/30 s PC values and clinical periodontal parameters indicate a positive relationship between disease severity and protein oxidation. In addition, GCF PC concentration values demonstrated a weak negative relationship with clinical parameters. In sum, the association of GCF PC total findings with clinical parameters was more prominent than the GCF concentration findings. The correlation analysis showed that the GCF PC total values were more closely related to the severity of the disease, while the GCF PC total values of both periodontitis groups increased compared with periodontal health. The increasing PC total levels in GCF than in serum and saliva suggested that protein oxidation was locally more prominent in GCF in periodontitis patients.

Oxidative stress and the severity of periodontitis are strongly linked (Baltacioglu et al., 2014a, 2014b). For this reason, the lower levels of GCF PC in the GAP group with severe destruction compared to the CP group suggests that different mechanisms of protein oxidation are active. Similarly, this theory is supported by the fact that there are lower saliva PC levels in the GAP group compared to the CP group. Salivary components are important determinants of oral health status. GCF components are also washed out from the periodontal pocket into saliva, and may therefore be mirrored in the salivary composition (Wu et al., 2009).

According to the evaluation of the findings of our present studies together, although there is consensus that PC levels are indicative of oxidative stress, it seems indisputable that there are some dark spots in protein oxidation, especially between two periodontitis groups. Several factors such as the

| Table 3 Correlations among serum, saliva and GCF PC levels and the clinical periodontal parameters in all participants. |
|---|---|---|---|---|
|  | r | p  | r | p  |
| PD-GCF PC conc | –0.197 | 0.001 |  |  |
| PD-GCF PC/ 30 s | 0.539 | 0.001 | 0.477 | 0.001 |
| CAL-GCF PC conc | –0.228 | 0.017 | –0.288 | 0.002 |
| CAL-GCF PC/ 30 s | 0.504 | 0.001 | 0.372 | 0.001 |
| GI-GCF PC conc | –0.244 | 0.010 | sa PC-GCF PC conc | 0.205 | 0.032 |

PD, probing depth; CAL, clinical attachment level; GI, gingival index; GBI, gingival bleeding index; PI, plaque index; sa, saliva; GCF, gingival crevicular fluid; 30 s, 30 s; conc, concentration; r, Pearson’s correlation coefficient.
nature of the oxidant, protein composition, the proximity of the metals to the target proteins could play a role in determining the degree of protein alteration (Baraibar et al., 2013). Furthermore, in various immunoinflammatory diseases in which oxidative stress plays a role in the etiology including periodontitis, proteins in different tissues are found to have different proportions and structure (Baraibar et al., 2013; Bostanci and Bao, 2017; Huynh et al., 2015). Current proteomic investigations have shown that gingivitis, chronic and aggressive periodontitis have different protein profiles from periodontal health, depending on immunoinflammatory (Bostanci and Bao, 2017; Huynh et al., 2015; Wu et al., 2009). When the proteomic profile of whole unstimulated saliva of GAP subjects was compared with the proteomic profiles of the healthy controls, 11 differential proteins were noted (Wu et al., 2009). Furthermore, in the light of the recent research, proteins targeted by oxidation were limited, which indicates that some proteins are more susceptible to oxidation. This “Oxi-proteome” is a potential molecular substratum for many cellular dysfunctions (Baraibar et al., 2013). Considering the above findings, we think that there may be some other factors playing a role in lower PC level in GAP of different proteomic profiles and oxi-proteome in serum, saliva and GCF. Therefore, further studies need to examine the proteomic profile along with protein oxidation.

Along with the above findings, recent research has shown different theories about protein carbonylation. As known, two chief categories of protein oxidative modifications, reversible and irreversible oxidation, are noted. These modifications could be triggered by ROS or reactive nitrogen species (RNS). Even though carbonylation and nitration might prove to have negative effects the target proteins, research suggests that under stress conditions such modifications could prove to be facilitative for cellular function (Cai and Yan, 2013). According to this theory, due to the relatively lower saliva and GCF PC levels in the GAP group, compared to the CP one, it is conceivable that the possible positive effects of PC were not utilized in GAP. Therefore, this situation was reflected in the destruction.

5. Conclusion

In sum, in this study, systemic (serum) PC levels showed no significant difference in comparison of periodontitis groups and periodontal health, while local PC (saliva and GCF) increased in CP compared to GAP and periodontal health, and generally lowest values were observed in GAP group. An increase in protein carbonylation in CP may be considered as an indicator of oxidative stress. However, findings showing that there is no difference compared to periodontal health or there are lower PC levels in GAP, emphasize on different proteomic profile and oxi-proteome.

Proteomic technologies have opened up new avenues into understanding the processes of inflammation and tissue destruction, which in turn heightened our awareness of periodontal infections (Baraibar et al., 2013; Bostanci and Bao, 2017). Recent studies indicate beneficial effects of protein oxidation. Therefore, proteomic identification of reversibly oxidized proteins might yield fruitful to help see their protective effects. As a result, it would be beneficial to conduct further studies examining proteomic profiling in chronic and aggressive periodontitis together with protein oxidation and other oxidative stress parameters, in order to elucidate the etiopathogenic mechanisms of both disease groups.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

The study protocol was examined by the Karadeniz Technical University Faculty of Medicine Ethics Committee, Trabzon, Turkey and approved.

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