Salivary Oxidative Variables Affect the Severity of Periodontal Disease in a Geriatric Population

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Abstract

Background: To evaluate the periodontal status and to investigate the possible role of some antimicrobial proteins and enzymes, and the oxidant/antioxidant balance in saliva of a geriatric population with different periodontal diseases. Assessments should be made about periodontal health, while preventive measures and treatment options/protocols should be developed for the elderly population in our country and in the world.

Methods: Eighty participants aged ≥ 65 years were included and their periodontal parameters were recorded. The salivary samples of this population with gingivitis (Group 1, n = 21) and periodontitis (Stage III periodontitis (Group 2, n = 23), and Stage IV periodontitis (Group 3, n = 36)) were investigated regarding the presence and levels of some parameters (lysozyme, lactoferrin, lactoperoxidase (LPO), sIgA), and total oxidant and antioxidant status (TOS and TAS) were analyzed with ELISA.

Results: Lactoferrin, sIgA, TAS, and OSI did not present statistically significant differences among the groups (P > 0.05). Lysozyme was similar in Group 1 and Group 2 (P > 0.05), but lower in both of these groups compared to Group 3 (P < 0.05). Linear discriminant factor function analysis revealed that salivary LPO, sIgA and TAS levels can discriminate the groups from each other. Strong correlations were found between lysozyme, LPO, and lactoferrin with periodontal parameters (p < 0.05).

Conclusions: It can be argued that the oxidative mechanism plays a role in the progression and severity of periodontitis in the study population of the present study.

Keywords

Elderly, Saliva, Lysozyme, Lactoferrin, Lactoperoxidase, Secretory immunoglobulin A, Oxidative stress

Introduction

The projection of global aging reported an increase to nearly 1.5 billion in 2050 [1]. Demographic changes related to old age occur in parallel to the rest of the world in our country. In Turkey: The elderly population ratio was estimated to increase to 20.8% in 2050 [2].

As a result of the aging process anatomical changes occur in major and minor salivary glands [3]. Human and animal studies show atrophy of the acinar cells occurs and normal gland parenchyma transforms to fibrous and/or adipose tissue with aging [4]. However, there are different opinions in the literature about salivary gland functions and saliva production in the elderly. In comparisons made according to age about whether there is a change in salivary flow rate (SFR) and amount with no drugs used. There are studies reporting that SFR decreases by half with age [5]. Salivary gland function and saliva production do not change under similar conditions and the decrease in SFR is not a result of normal aging [6].
Saliva contains many substances that originate from the host and bacteria such as cationic peptides, digestive enzymes, neuropeptides, mucin, surfactant protein A, beta-2 microglobulin, fibronectin, metal ion chelators, cystatin, peroxidases and lysozyme [7]. Lysozyme is found in saliva, milk, tears and gingival crevicular fluid (GCF). Has antimicrobial activity by hydrolyzing the polysaccharides of the bacterial cell membrane [8]. While lysozyme levels in GCF were not reported to differ between a chronic periodontitis and periodontally healthy control group [9]. It was significantly higher in the aggressive periodontitis group than in the chronic periodontitis and periodontally healthy groups (respectively) [10].

Lactoferrin exerts bactericidal or bacteriostatic effect against microorganisms that need iron to survive by binding to free iron in saliva [11]. Salivary levels of lactoferrin were reported to be increased in chronic periodontitis [12].

Secretory immunoglobulin A (sIgA) plays the main role in adaptive immunity [13]. Salivary IgA interferes with microbial adhesion by binding to different molecular components with its secretory IgA (sIgA) form [14].

Lactoperoxidase (LPO) acts as a catalyst together with hydrogen peroxide (H_{2}O_{2}) for the oxidation of the thiocyanate ion in saliva to hypothiocyanate a powerful antibacterial agent [15]. As a result of the use of peroxidase cells and proteins are protected from the toxic and oxidant effects of H_{2}O_{2} [16].

The salivary antioxidant system has gained increased attention nowadays. The production of reactive oxygen species by neutrophils with periodontitis formation results in tissue damage when not balanced by the antioxidant defense system. Oxidative stress related to periodontitis was investigated using total antioxidant and oxidant status (TAS and TOS), oxidative stress index (OSI) and increases in systemic and local oxidative stress and decrease in antioxidant capacity were reported by studies [17,18].

Although the details of how mechanistic changes in immune functioning related to salivary gland function, saliva production and salivary content are not clear [19]. Age-related changes may increase the risk of tooth decay, gingival inflammation and periodontal disease. When the saliva lactoferrin, LPO, lysozyme and sIgA levels of old and young individuals were compared, many conflicting and different results were obtained [20-22].

However, according to a search we conducted in the literature, there are no studies evaluating the changes in saliva content with different periodontal conditions among elderly people. The aim of this study is to test hypothesis for accuracy that the periodontal disease type (gingivitis, periodontitis) and severity (Stage 3 and Stage 4 for periodontitis) are related to differences in some antimicrobial proteins, enzymes and oxidant/antioxidant balance.

Methods

The Local Ethics Committee approved the study (04.10.2017; no. 171). The study was conducted in accordance with the ethical standards stated in the Declaration of Helsinki (revision in 2013). Participation in the study was based on volunteering and informed consent forms were obtained from all of the patients.

Participants

Eighty consecutive individuals who volunteered were ≥ 65-years-old who applied to the Geriatrics Clinic in Suleyman Demirel University (SDU) Faculty of Dentistry between April 2017 and December 2018. Who fulfilled the following criteria and the criteria of the targeted periodontal disease subgroups mentioned below were included in the study.

Edentulous individuals; individuals with a history of periodontal treatment in the last six months antibiotic and/or anti-inflammatory drugs in the last three months with acute periodontal diseases. Who had rheumatologic and/or autoimmune diseases, malignancies and individuals who refused to participate were excluded from the study.

The sociodemographic characteristics of the study population such as gender, monthly income, education level, etc. were recorded. The existing systemic diseases and drugs used by the participants their blood pressure calculations of body mass index (BMI) and waist to hip ratio (W/H) were recorded.

Clinical dental and periodontal parameters

Dental examinations were completed by the same qualified examiner (ARI). Within the scope of the examination, the number of teeth, the presence of carries, root canal therapies, restored teeth and removable prosthetic devices were also recorded. The periodontal parameters namely the plaque index (PI) [23] bleeding on probing (BOP) [24] clinical attachment loss (AL) and probing pocket depth (PPD) were recorded of the present teeth. The periodontal inflamed surface area (PIA) and periodontal epithelial surface area (PESA) scores were also calculated [25]. The study groups were composed according to the updated classification for periodontal and peri-implant diseases and conditions [26] as follows: Group 1: Generalized gingivitis with reduced periodontium [27] Group 2: Stage III periodontitis and Group 3: Stage IV periodontitis [23].

Sampling of saliva

Saliva (unstimulated whole) was collected between 9 and 10 am over 5 minutes duration by passive drooling into sterile plastic tubes by all patients according to the method of Navazesh and Kumar (2008) [27].

Biochemical analysis

The saliva samples were stored -80 °C until assay and
centrifuged (15 minutes, 10,000 g). Quantitative measurement of lactoferrin (E-EL-H5200), LPO (E-EL-H1362), lysozyme (E-EL-H1869) and slgA (E-EL-H1275) from the supernatants of saliva samples was performed using enzyme-linked immunosorbent assay (ELISA) kits Elabscience Biotechnology Co. Ltd. Wuhan, China). The sensitivity of the kits were 0.19 ng/ml, 0.1 ng/ml, 0.13 pg/ml, 0.75 ng/ml, 0.19 ng/ml and 1.05 μg/ml Respectively. Each kit had seven standards for the calibration graph. All standards and samples were paired to obtain optical density data corresponding to these concentrations. At the end of the procedure, the optical density-concentration graph of the standards was plotted and the concentrations of all samples were calculated using this calibration graph. Lactoferrin samples were diluted 1:700 times, the results were multiplied by 700 and expressed in units of ng/ml. LPO samples were diluted 1:100 times, the results were multiplied by 100 and expressed in units of ng/ml. SlgA samples were diluted 1:10,000 times, the results were multiplied by 10,000 and expressed in ug/ml. Lysozyme samples were used directly and the results are expressed in units of ng/ml.

Commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used to determine the total antioxidant and oxidant status (TAS and TOS) levels spectrophotometrically on a biochemistry analyzer (Beckman Coulter, AU 5800, Beckman Coulter, Brea, USA) by the application of the Erel methods [28,29].

Statistical evaluation

The data were examined to determine whether they abided by the preconditions for parametric tests (Box\' M test and Kolmogorov-Smirnov test). When the preconditions were confirmed, the data analysis began with one-way analysis of variance, then Tukey multiple comparison tests and when the preconditions were not met. The Kruskal-Wallis test was performed followed by Bonferroni-Dunn test. The chi-square independent test was used in cross tabulation tables to analyze the categorical demographic data. The discriminant function analysis was completed to classify the groups related to periodontal health and disease using the investigated periodontal and salivary parameters correctly with no or minimum error. Spearman rank correlation analyses were used to determine any correlations. Commercial statistical software (IBM SPSS Statistics for Windows, version 23, IBM Corp, Armonk, NY, USA) was used for all statistical analyses (P < 0.05).

Results

In Table 1: Characteristics of the study population are presented. (Table 1) The subgroups based on current periodontal disease status (gingivitis, Stage III periodontitis and Stage IV periodontitis) did not have statistically significant differences regarding sociodemographic, anthropometric, medical and dental variances (P > 0.05). NT was significantly lower in the Stage IV periodontitis group than the gingivitis and Stage III periodontitis groups (mean ranks: Gingivitis group: 47.76, Stage III periodontitis group: 55.54, Stage IV periodontitis group: 26.65; P < 0.05).

Significant differences among the groups were determined regarding some periodontal variances (Table 2). BOP, PISA and PESA values were not significantly different between the periodontitis groups (P > 0.05) but were significantly higher than the gingivitis group (P < 0.05; Table 2). PPD and CAL values were significantly different among the three groups (P < 0.05). PI values were similar in the gingivitis and Stage III periodontitis groups (P > 0.05) but were lower than the Stage IV periodontitis group in both of these groups (P < 0.05; Table 2). The gingivitis group had significantly higher SFR than both of the periodontitis groups (Stage III and Stage IV; P < 0.05). Stage III and Stage IV periodontitis patients had similar SFR values (P > 0.05).

The gingivitis and Stage III periodontitis groups displayed similar salivary lysozyme levels (P > 0.05; Table 3) but in both of these groups salivary lysozyme levels

| Parameters                                  | Values                        |
|---------------------------------------------|-------------------------------|
| BMI (kg/m²) (mean ± SD)                     | 28.05 (19.81-40.15)           |
| W/H                                         | 0.91 (0.76-1.09)              |
| Systemic Disease (n (%))                    |                               |
| Present                                     | 55 (68.75)                    |
| Absent                                      | 25 (31.25)                    |
| Distribution of systemic disease (n (%))    |                               |
| CVD                                         | 8 (10)                        |
| DM                                          | 13 (16)                       |
| CVD & DM                                    |                               |
| Systolic blood pressure (mmHg) (mean (min-max)) | 129.63 (100-200)             |
| Diastolic blood pressure (mmHg)             | 79.75 (60-100)                |
| NT                                          | 20.81 ± 5.45                  |
| Teeth brushing frequency                    |                               |
| Seldom                                      | 24 (30)                       |
| 2/3 in a week                               | 19 (23.8)                     |
| Every day                                   | 37 (46.2)                     |
| SFR (ml/min)                                | 0.36 ± 0.24                   |
| Number of missing teeth (mean ± SD)         | 7.05 ± 5.36                   |
| Number of decayed teeth (mean ± SD)         | 1.28 ± 2.13                   |
| Number of filled teeth (mean ± SD)          | 1.51 ± 2.18                   |
| Number of root/canal therapy (mean ± SD)    | 0.92 ± 1.63                   |
| DMFT                                        | 10.76 ± 5.72                  |

BMI: Body mass index; CVD: Cardiovascular disease; DM: Diabetes Mellitus; DMFT: Decayed/missed/filled teeth; NT: Number of existing teeth; SD: Standard deviation; SFR: Salivary flow rate; W/H: waist to hip ratio.
Table 2: The periodontal parameter values and salivary flow rate of the study groups.

| Groups                      | Parameters       | Gingivitis (n = 21) | Stage III Periodontitis (n = 23) | Stage IV periodontitis (n = 36) |
|-----------------------------|------------------|---------------------|----------------------------------|---------------------------------|
|                             | PPD (mm)         | Mean ± SD           | Min - Max                        |                                 |
|                             |                  | 1.63 ± 0.34<sup>a</sup> | 1.47-1.79                        | 2.86 ± 0.60<sup>b</sup>        |
|                             |                  | 1.01-2.73           |                                  | 3.40 ± 0.80<sup>c</sup>        |
|                             | AL (mm)          | Mean ± SD           | Min-Max                          |                                 |
|                             |                  | 3.09 ± 0.80<sup>a</sup> | 1.67-5.12                        | 4.57 ± 1.22<sup>b</sup>        |
|                             |                  | 1.67-5.12           |                                  | 5.76 ± 1.42<sup>c</sup>        |
|                             | PI               | Mean ± SD           | Min-Max                          |                                 |
|                             |                  | 1.79 ± 0.69<sup>a</sup> | 1.48-2.11                        | 1.97 ± 0.72<sup>a</sup>        |
|                             |                  | 0.75-3.00           |                                  | 1.64-3.31                       |
|                             | BOP %            | Mean rank           | 28.41<sup>a</sup>               | 37.78<sup>b</sup>              |
|                             |                  | 19.05<sup>a</sup>   | 47.30<sup>b</sup>               | 48.67<sup>b</sup>              |
|                             | PISA (mm<sup>2</sup>) | Mean rank  | 28.41<sup>a</sup>               | 37.78<sup>b</sup>              |
|                             |                  | 19.05<sup>a</sup>   | 47.30<sup>b</sup>               | 48.67<sup>b</sup>              |
|                             | PESA (mm<sup>2</sup>) | Mean ± SD            | 807.32 ± 319.02<sup>a</sup> | 1693.25 ± 513.16<sup>b</sup> |
|                             |                  | 662.11-952.54       | 471.35-1915.16                   | 1395.71 ± 576.10<sup>b</sup> |
|                             |                  | 336.66-1635.22      | 745.88-2735.59                   | 1200.78-1590.63                 |
|                             | SFR (ml/min)     | Mean rank           | 46.93<sup>a</sup>               | 33.63<sup>b</sup>              |
|                             |                  | 19.05<sup>a</sup>   | 47.30<sup>b</sup>               | 48.67<sup>b</sup>              |

PPD: Probing pocket depth; AL: Clinical attachment loss; PI: Plaque index; BOP: Bleeding on probing; PISA: Periodontal inflamed surface area; PESA: Periodontal epithelial surface area. SD: Standard deviation; SFR: Salivary flow rate; CI: Confidence interval for mean; L: Lower Bound; U: Upper bound; Min: Minimum; Max: Maximum. Different Lowercase Letters indicate Statistically Significant Differences (P < 0.05; Kruskal-Wallis; ANOVA Analysis).

Table 3: The salivary antibacterial parameter values of the study groups.

| Groups                      | Parameters       | Gingivitis (n = 21) | Stage III Periodontitis (n = 23) | Stage IV periodontitis (n = 36) |
|-----------------------------|------------------|---------------------|----------------------------------|---------------------------------|
|                             | Lysozyme (ng/mL) | Mean rank           | 30.57<sup>a</sup>               | 40.13<sup>a</sup>              |
|                             | Lactoferrin (ng/mL) | Mean rank  | 39.90                           | 42.35                          |
|                             | LPO (ng/mL)      | Mean rank           | 55.62<sup>a</sup>               | 30.39<sup>b</sup>              |
|                             | sIgA (µg/mL)     | Mean ± SD           | 181.09 ± 38.02<sup>a</sup>      | 178.11 ± 41.95<sup>b</sup>     |
|                             | TAS (mmolTroloxequiv./L) | Mean ± SD  | 0.72 ± 0.37<sup>a</sup>        | 0.85 ± 0.44                    |
|                             | TOS (umol H<sub>2</sub>O<sub>2</sub> equiv./L) | Mean ± SD      | 27.56 ± 41.94<sup>a</sup>      | 32.65 ± 49.56<sup>b</sup>     |
|                             | OSI               | Mean ± SD           | 3.57 ± 5.72<sup>a</sup>        | 3.01 ± 3.18                    |

SigA: Salivary immunoglobulin A; LPO: Lactoperoxidase; Min: Minimum; Max: Maximum; OSI: Oxidative stress index; SD: Standard deviation; TAS: Total antioxidant status; TOS: Total oxidant status. Different Lowercase Letters Indicate Statistically Significant Differences (P < 0.05; Kruskal - Wallis; ANOVA Analysis).
were significantly lower than the Stage IV periodontitis group (P < 0.05; Table 3). The Stage III and IV periodontitis groups had similar LPO levels (P > 0.05; Table 3). Their LPO levels were significantly lower than the gingivitis group (P < 0.05). None of the other salivary parameters (lactoferrin, slgA, TAS, TOS and OSI) showed significant differences among the groups (P > 0.05; Table 3).

Discriminant function analysis was performed to classify the groups related to periodontal disease using the investigated periodontal and salivary parameters correctly with no or minimum mistakes. After discriminant analysis, the discrimination of groups using discrimination functions revealed that the groups were differentiated with 83.8% success using the variances for PPD, NT, LPO, and TAS. The discrimination percentages were found as follows: 20 from 21 in the gingivitis group (95.2%), 20 from 23 in the Stage III periodontitis group (87%), and 27 from 36 in the Stage IV periodontitis group (75%). The functions to discriminate the patients into groups are shown in Table 4 and Table 5 the classification results of the discriminant analysis are given.

The correlations (between periodontal and salivary parameters) which were statistically significant are presented in Table 6.

### Table 4: Linear discriminant function for groups.

| Groups                                   | Parameters                  | Gingivitis (n = 21) | Stage III periodontitis (n = 23) | Stage IV periodontitis (n = 36) |
|------------------------------------------|-----------------------------|---------------------|----------------------------------|---------------------------------|
| Constant                                 | -32.240                     | -46.904             | -45.565                          |
| NT                                       | 1.306                       | 1.360               | 1.005                            |
| PPD (mm)                                 | 6.493                       | 11.221              | 12.575                           |
| LPO (ng/mL)                              | 0.002                       | -0.010              | -0.011                           |
| slgA (ug/ml)                             | 0.083                       | 0.106               | 0.119                            |
| TAS (mmol Trolox equiv./L)               | 8.628                       | 12.655              | 11.929                           |

Sig A: Salivary Immunoglobulin A; LPO: Lactoperoxidase; NT: Number of existing teeth; PPD: Probing pocket depth; TAS: Total antioxidant status.

### Table 5: The classification results of the discriminant analysis (N = 80).

| Groups | Predicted Group Membership | Gingivitis | Stage III periodontitis | Stage IV periodontitis | Total |
|--------|----------------------------|------------|-------------------------|------------------------|-------|
|        | Original Count             |            |                         |                        |       |
|        | Gingivitis                 | 20         | 1                       | 0                      | 21    |
|        | Stage III periodontitis    | 1          | 20                      | 2                      | 23    |
|        | Stage IV periodontitis     | 1          | 8                       | 27                     | 36    |
|        | %                          | 95.2       | 4.8                     | 0.0                    | 100.0 |
|        | Stage III periodontitis    | 4.3        | 87.0                    | 8.7                    | 100.0 |
|        | Stage IV periodontitis     | 2.8        | 22.2                    | 75.0                   | 100.0 |

### Table 6: The statistically significant correlations between investigated parameters (N = 80).

| Parameters          | rho     | p      |
|---------------------|---------|--------|
| Lactoferrin (ng/mL)-LPO (ng/ml) | 0.405   | 0.001  |
| Lactoferrin (ng/mL)-TAS (mmol Trolox equiv./L) | 0.586   | 0.001  |
| Lactoferrin (ng/mL)-TOS (umol H₂O₂ equiv./L) | 0.500   | 0.001  |
| Lactoferrin (ng/mL)-OSI | 0.331   | 0.003  |
| LPO (ng/mL)-slgA (µg/mL) | 0.250   | 0.026  |
| LPO (ng/mL)-TOS (umol H₂O₂ equiv./L) | 0.227   | 0.043  |
| LPO (ng/mL)-OSI | 0.269   | 0.016  |
| TAS (mmol Trolox equiv./L)-TOS (umol H₂O₂ equiv./L) | 0.451   | 0.001  |
| TOS (umol H₂O₂ equiv./L)-OSI | 0.847   | 0.001  |
| PPD (mm)- Lysozyme (ng/mL) | 0.383   | 0.001  |
Discussion

This study was conducted to evaluate the periodontal conditions of geriatric individuals living in the southern Mediterranean, who applied to a University Geriatric Clinic to investigate the role of the antibacterial components of saliva in the pathogenesis of periodontal disease in this particular population. All of the individuals in our study population had the periodontal diseases of gingivitis and periodontitis. The absence of any periodontally healthy individuals among the groups applying to the clinic and meeting the criteria for inclusion at the time of the study emphasizes the importance of developing preventive and therapeutic strategies for oral and periodontal health in this particular patient group in our country.

In the present study biochemical examinations were performed on saliva with the hypothesis that there will be differences in some antimicrobial proteins, enzymes and the oxidant/antioxidant balance in different periodontal diseases. The results of the present study partially confirmed our hypothesis and it was thought that the oxidative mechanism plays a role in the progression of periodontitis in the study population.

Although there is a rich microbial environment in the oral cavity about 45 known antimicrobial enzymes which form the host defense mechanism and exist in saliva protect the mucosa from infections prevent the proliferation of microorganisms and are secreted by oral epithelial cell, salivary glands and neutrophils [7]. These enzymes are involved in generating the initial response in innate immunity against invading pathogens [30]. In the present study some of these known antimicrobial enzymes and proteins namely Lysozyme, lactoferrin, LPO and slgA were evaluated.

In the present study gingivitis and Stage III periodontitis groups had similar salivary lysozyme levels with both groups found to have values significantly lower than the Stage IV periodontitis group. Similar results were obtained in a study [31]. Which included a population in the age spectrum between 39-69 years that was not conducted to evaluate the situation in geriatric individuals. Surna, et al., [8] and Syrjänen, et al. [9] also reported higher lysozyme levels or activity in the saliva and/or GCF samples of periodontitis group when compared to periodontally healthy and gingivitis groups supporting the results of our study. The increase in lysozyme may be an indicator of the effort to return to homeostasis with antibacterial activity as the severity of periodontitis increases. Another important finding that should be noted is that the salivary level of lysozyme in gingivitis is similar to the Stage III periodontitis group. Considering systemic involvement in elderly individuals (significant saliva parameters and PISA and PESA correlations), gingivitis should be considered as important as periodontitis for preventive and therapeutic protocols. In the study conducted by Syrjänen, et al. [9] lysozyme levels in GCF did not differ between chronic periodontitis and the periodontally healthy control group but in the study of Friedman, et al. [10] it was observed that lysozyme and lactoferrin were significantly higher in aggressive periodontitis than in the healthy group. In the present study, salivary lactoferrin levels did not show significant differences among the three groups and there was no periodontally healthy group. The findings of our study regarding lactoferrin do not confirm our hypothesis, similar to the inconsistent results in the literature.

Another investigated variable LPO was found to be significantly higher in the gingivitis group than the two periodontitis group, the severity of periodontitis did not affect the salivary LPO levels in both of the periodontitis groups with no difference observed between them. Studies about the use of lozenges containing LPO in periodontal disease [32-34] (two studies [32,33] conducted with elderly participants) markedly emphasize the importance of LPO for antibacterial activity in the saliva of the elderly. In the present study LPO also had significant correlations with TOS, slgA and PESA.

Salivary IgA had no significant differences among the groups. However, it was able to discriminate the periodontal disease groups along with the other salivary variances of LPO and TAS. In our study TOS, TAS and OSI had no significant difference between the groups but had significant positive correlations with lactoferrin and

|                  | NT- Lactoferrin (ng/mL) | CAL (mm)- Lysozyme (ng/mL) | PESA (mm²)- Lactoferrin (ng/mL) | PESA (mm²)-LPO (ng/ml) | PESA (mm²)- Lysozyme (ng/mL) | PISA (mm²)- Lysozyme (ng/mL) | SFR (mL/min)-PPD (mm) | SFR (mL/min)-AL (mm) | SFR (mL/min)- Lysozyme (ng/mL) |
|------------------|------------------------|---------------------------|-------------------------------|------------------------|-------------------------------|-------------------------------|------------------------|-------------------|------------------------|
|                  | 0.233                  | 0.324                     | -0.308                        | -0.225                 | 0.299                         | 0.369                         | -0.339                 | -0.276             | -0.265                 |
|                  | 0.038                  | 0.003                     | 0.005                         | 0.045                  | 0.007                         | 0.001                         | 0.004                  | 0.021              | 0.026                  |

AL: clinical attachment loss, IgA: immunoglobulin A, LPO: lactoperoxidase, slgA: salivary immunoglobulin A. SD: standard deviation, SFR: salivary flow rate, OSI: oxidative stress index, TAS: Total antioxidant status, TOS: Total oxidant status. PPD: probing pocket depth, PISA: periodontal inflamed surface area, PESA: periodontal epithelial surface area, (Spearman correlation analysis, P < 0.05).
Another important finding in our study is the decrease in SFR in periodontitis groups compared to gingivitis. Groups with periodontitis were similar to each other but exhibited significantly lower SFR than the group with gingivitis. Significant positive correlations were found between SFR with PPD, SFR, AL and a negative correlation was found between SFR and lysozyme. In another study evaluating whether the changes in salivary content are related to the amount of saliva, negative significant correlations were reported between Lysozyme, stimulated SFR, total IgA and lactoferrin [22].

LPO concentration was highest in the oldest group. It was concluded that age does not affect the antimicrobial agent concentrations in saliva of this population who do not use drugs [22].

Unfortunately, 68.75% individuals in the study population were individuals with systemic disease and used prescription drugs in the present study. When they are divided into groups according to their periodontal health, sociodemographic characteristics and presence of systemic disease did not present significant differences among the groups and these characteristics had no significant correlations with biochemical parameters (data not shown). However, it cannot be said that the biochemical parameters we examined were not affected by the drugs used by the patients. For this reason, these issues should also be considered while making comments regarding our findings. Further studies about this subject in larger populations including periodontal-ly and systemically healthy geriatric patients may provide clearer findings.

It is difficult to mention a certain linear increase/decrease in studies where salivary antibacterial enzymes are compared between different periodontal conditions and pre- and post-periodontal treatment values regardless of age. The lack of comparative results for salivary antibacterial parameters in the elderly population according to different periodontal conditions (gingivitis and periodontitis groups separated according to severity) in the literature makes it difficult to interpret our findings by comparison. Another problem with making clearer comments regarding the results is that the definition of periodontal diseases varies in existing studies.

Despite important findings, our study has some limitations. Firstly, a cause-and-effect relationship cannot be determined with this cross-sectional study. Although our hypothesis was not established for this purpose, research should be planned with various designs for a national health plan as supported by our findings. Secondly, the population we evaluated in our research about elderly individuals does not represent the elderly population in our country. The lack of periodontally healthy individuals and edentate patients should be taken into consideration in this regard. Since the participants were classified according to periodontal condition individuals with 10 or more teeth were evaluated and the prevalence of edentulousness was not evaluated. Therefore, the cause of tooth loss (caries or periodontal) cannot be determined with our findings. However, the purpose of our study was not to determine the cause of edentulousness.

The strengths of our work should also be noted with these limitations. In our country, the number of studies examining periodontal conditions of elderly individuals is low and to our knowledge there are no studies examining salivary parameters belonging to different periodontal disease groups in elderly individuals. Our study is a first in our country from this aspect.

Since classification and analysis were made according to the severity of periodontal disease in the present study gingivitis as well as periodontitis was emphasized with both clinical parameters and biochemical parameter levels in terms of systemic inflammatory load in an attempt to show inflammatory surface area regarding gingival/periodontal inflammation with PISA measurements. This is an extremely important finding in the relationship between systemic diseases and periodontal disease especially in elderly individuals with many systemic diseases.

Conclusions

Although the decrease in salivary antibacterial activity in elderly individuals was not clearly demonstrated in current studies the effect of this possible situation (decrease in SFR - for various reasons, reduction in saliva antibacterial components) on oral, dental and periodontal health is worth detailed research. According to the findings in our study, differences were observed in elderly individuals depending on the type and severity of periodontal disease and some variables (LPO, sIgA and TAS) were found to be successful in differentiating periodontal disease in this population.

Author Contribution Statement

All authors have made substantial contributions: ARI: Data curation; Investigation; Methodology; Writing - original draft preparation; ŞÇ: Formal analysis; Writing - original draft preparation; FBŞ: Formal analysis; Methodology; Writing - original draft preparation; Writing - review & editing; ÖK: Software; Formal analysis; Methodology; Writing - original draft preparation; Writing - review & editing; ZYA: Conceptualization; Supervision; Funding acquisition; Investigation; Methodology; Writing - original draft preparation; Writing - review & editing.

Acknowledgments

This study was supported by the Coordination Unit of Scientific Investigations, Süleyman Demirel University, Isparta, Turkey (project number: TDH-2018-5959).

Işık et al. J Geriatr Med Gerontol 2021, 7:109

DOI: 10.23937/2469-5858/1510109

ISSN: 2469-5858
Conflict of Interest

The authors of this study report no conflicts of interest related to this study.

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ISSN: 2469-5858
DOI: 10.23937/2469-5858/1510109

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