Biological Trace Element Research (2020) 197:35–42
https://doi.org/10.1007/s12011-019-01977-z

The Association Between Some Macro and Trace Elements in Saliva and Periodontal Status

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Received: 2 September 2019 / Accepted: 4 November 2019 / Published online: 17 December 2019

Abstract

Changes in the macro and trace element composition of saliva might be indicative for pathological changes in periodontal tissues. However, there is a lack of evidence in the literature showing associations between mineral elements and periodontal status. The aim of this study was to determine whether such associations occur. Totally, 190 systemically healthy non-smoker participants (mean age 32.2 ± 6.02; 50 periodontally healthy, 50 gingivitis, 50 chronic periodontitis, and 40 aggressive periodontitis individuals) were included in this cross-sectional study. Salivary levels of some macro and trace elements were measured by using inductively coupled plasma mass spectrometry (ICP-MS). Kruskal-Wallis's test was used for statistical analysis. Statistically significant differences were found in sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), vanadium (V), chromium Cr), manganese (Mn), iron (Fe), rubidium (Rb), strontium (Sr), and selenium (Se) concentrations among the groups. Significant increases in the essential minerals Na, Mg, K, Ca, Fe, and Se occurred in both periodontitis groups when compared to the gingivitis and periodontally healthy groups. Lower Se, Sr, Fe, Mn, and V concentrations were found in the aggressive periodontitis group than in the chronic periodontitis group. The results of this study demonstrated that assessment of mineral element concentrations in saliva might be useful in assessing periodontal health and disease. However, further studies are required to determine whether the change in a specific mineral element is the result of periodontal disease or is involved in its pathogenesis.

Keywords Saliva · Periodontitis · Gingivitis · Trace elements · Elements

Introduction

Periodontitis is a polymicrobial infectious disease of the periodontium that is associated with pathogenic microbiota in the subgingival biofilm. Although local, systemic, environmental, and genetic factors play a role in the etiology of periodontal disease, the primary etiological factor is known to be microbial dental plaque and its products [1]. Periodontitis is common among the general population, and it is known to adversely affect systemic health and quality of life [2]. The host response to the microbial flora has a strong impact on the development of the disease because it exerts a destructive effect on the periodontal tissues [3]. As the disease develops, collagen fibrils surrounding and supporting the periodontium are destroyed; gingival attachment loss on the root surface occurs with apical migration of the sulcus epithelium, development of deep pocket, and/or gingival recession, which results in severe bone loss and eventual tooth loss [4].

In periodontology and implant surgery, the traditional clinical criteria, such as probing pocket depths, clinical attachment levels, and radiographic alveolar bone levels, are often inadequate to diagnose the active disease, to assess the response to treatment, and to measure a patient's susceptibility to future disease progression. Saliva has been indicated as a valuable source of clinical information concerning oral and systemic health because it contains the biomarkers of inflammation and bone loss in periodontal disease [5]. A qualitative change in the composition of these biomarkers such as proteins of host origin (i.e., enzymes, immunoglobulins), phenotypic markers (epithelial keratins), host cells, hormones (cortisol), bacteria and bacterial products, volatile compounds, and ions may serve as indicators of the initiation and progression of

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periodontal disease and assist with monitoring the efficacy of treatment [6, 7]. Importantly, the components that pass from the blood through passive diffusion, active transport, or extracellular ultra-filtration to the saliva and gingival crevicular fluid are easily available for analysis [8].

Both macro and trace minerals have essential roles in the host response to prevent the progression of chronic diseases such as periodontitis [9–13]. Mineral nutrients such as calcium (Ca), magnesium (Mg), iron (Fe), selenium (SE), and zinc (Zn) have different functions that affect immunoinflammatory and bone maintenance pathways [14]. The determination of their concentrations in saliva might be useful in assessing the progression of periodontal disease and the appraisal of periodontal treatment success [15, 16]. Mineral element levels in saliva have been measured by different methods such as atomic absorption spectrometry, thermal neutron activation analysis, gamma ray spectrometry, and most recently by inductively coupled plasma mass spectrometry (ICP-MS). Because ICP-MS is the method that allows the fast and accurate routine multi-element determination with improved sensitivity for biological samples [17], it was used in the present study to determine the salivary macro and trace element levels of individuals with different periodontal status to assess whether there is an association between some mineral concentrations in saliva and periodontal health, which could assist in the monitoring periodontal disease.

Materials and Methods

Study Design

In total, 190 systemically healthy, non-smoking participants (mean age 32.2 ± 6.02; 50 periodontally healthy control subjects (H), 50 patients with gingivitis (G), 50 with chronic periodontitis (CP), and 40 with generalized aggressive periodontitis (GAP)) referred by the Dentistry of Selcuk University Periodontology Department between 2014 and 2016 were included in this cross-sectional study. All participants prior to enrollment signed an informed consent form that included written information describing the nature of the study. The study protocol was approved by the Ethics Commission of Selcuk University Faculty of Dentistry Non-Interventional Studies (2014/02).

Study Population

For inclusion in this study, subjects had to be systemically healthy (excluding the case definition), have a minimum of 20 teeth, and be older than 18 years of age. Individuals were excluded if they had any systemic disease (liver and/or kidney dysfunction; diabetes; undergone organ transplant or cancer therapy; cardiovascular diseases) or had been treated previously for periodontal disease. Additional exclusion criteria included pregnancy or lactation, use of antibiotics or immunosuppressant medications within the last 6 months, need for antibiotics for infective endocarditis prophylaxis during dental procedures, or symptoms of acute illness.

Baseline evaluation of subjects included measurements of plaque index (PI), gingival index (GI), probing depth (PD) [18, 19], and clinical attachment level (CAL) using a periodontal probe (HuFriedy, Chicago, IL, USA). Also, a full mouth periodontal examination was performed at six locations per tooth (mesial-buccal, mid-buccal, distal-buccal, mesial lingual, mid-lingual, and distal-lingual) with the exception of the third molars. Medical and dental histories and exclusion criteria were reviewed prior to the periodontal examination. Healthy controls were categorized based upon a PD ≤ 3 mm and a lack of clinical gingival inflammation (no more than 10% of the sites with bleeding upon probing and absence of gingival redness/edema) and radiographic evidence of alveolar crestal bone loss [20]. Patients in the G group were defined as having GIs > 0, PDs ≤ 3 mm, and CALs ≤ 2 mm at ≥ 90% of teeth with no radiographic signs of alveolar bone loss caused by periodontal disease. Patients with sites presenting with CALs ≥ 3 mm, GIs > 0, and PPDs of ≥ 6 mm at multiple sites were defined as having CP. Patients with usually under 30 years of age were diagnosed with GAP if they demonstrated rapid attachment loss and bone destruction inconsistent with amounts of microbial deposits. Subjects with periodontitis and gingivitis were identified according to the 1999 international classification criteria [21].

Collection of Saliva

Unstimulated saliva samples were collected from each patient prior to clinical evaluation. Patients were instructed not to consume any type of food at least 1 h before saliva collection. Unstimulated saliva samples during a 5-min period was collected in a plastic cup. The total saliva collected was aspirated into a disposable 5 mL sterile syringe [22]. The amount of saliva in mL/min was recorded as the mean salivary flow rate. The unstimulated saliva (3 mL) was stored at − 80 °C until mineral element analyses were performed.

Analysis of Saliva Trace Elements Levels

Trace element concentrations in saliva samples were determined at the Selçuk University Dental Faculty Research Center. Inductively coupled plasma mass spectrometry (ICP-MS) is the method that the fast and accurate routine multi-element determination in biological samples has become possible due to improved sensitivity and robustness. The concentrations of mineral elements in the saliva samples were measured by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan DRC-e, PerkinElmer, Inc., Waltham,

MA, USA). After bringing to a temperature of + 4 °C, 10 mL of 65% nitric acid was added to a 0.5 to 1.0 g sample of saliva. All samples reacted homogeneously with the acid by 10 min. Specimens were then digested in a microwave oven (CEM Corporation, Mars 5, Matthews, NC, USA). at 1600 Watts, 100% power, at 160 °C for 40 min. After digestion, samples were placed in centrifuge cups and 50 ml ultrapure water were added before centrifuging at 1000–3000 rpm for 10 min to eliminate cellular debris and performing the ICP analysis.

A calibration curve was made by using 8 separate concentrations (1, 10, 50, 100, 250, 500, 1000, 2000 ppb) made from a standard solution for each of the determined elements. The average macro (Ca, Na, K, Mg) and micro (Fe, Zn, Mn, Cu, Mo V Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Ga, In, Li, Na, Ni, Pb, Rb, Se, Sr , Ti, U, V) element concentration in saliva were recorded in parts per billion and converted to parts per million (ppm) for the minerals focused upon in this report.

Statistical Analyses

Before statistical analyses, distributional properties of the data were evaluated using the *Anderson-Darling* test. Descriptive statistics were obtained. Groups were compared by using the *Kruskal-Wallis test* and *the Dunn test* was used for post hoc test analyses. The chi-square test of independence was used to determine whether there was a significant relationship between two categorical variables. A $p < 0.05$ value was accepted as statistically significant. All statistical analyses were performed by using the R statistical package [23].

Results

The demographic findings and clinical periodontal measurements, including minimum and maximum values, of the total 190 individuals are shown in Table 1. The age of the individuals in the chronic periodontitis group was significantly higher than the ages of the other groups ($p < 0.001$). There were no significant differences among the groups in body mass index (BMI).

Significant differences were found in all the clinical periodontal measures (Table 1). The decayed, missing and filled teeth (DMFT), PD, GI, and CAL values were significantly higher in the CP and GAP groups than in the H and G groups. There were no statistical differences in these variables between the CP and the GAP groups ($p > 0.05$). However, PI and GI values were significantly higher in the G than the H group. Only the GAP group exhibited significantly elevated PD values.

The salivary concentrations of minerals showing significant differences among the H, G, CP, and GAP groups are shown in Table 2. The elements Ag, Al, As, Ba, Be, Bi, Cd, Co, Cs, Cu, Ga, In, Ni, Pb, Ti, and U were below the detection threshold. No statistically differences were found in saliva zinc concentrations between the groups ($p > 0.05$). The

Table 1 Demographic variables, clinical periodontal measurements, and salivary flow rates of patients. Values are median (min.–max.)

	Groups				
	Healthy (H) ($n = 50$)	Gingivitis (G) ($n = 50$)	Chronic periodontitis (CP) ($n = 50$)	Generalized aggressive periodontitis (GAP) ($n = 40$)	p
Age (y)	28.0 (25.0–41.0)***	24.5 (20.0–56.0)***	43.5 (27.0–58.0)***	28.0 (20.0–35.0)***	$p < 0.001$*
BMI (kg/m^2)	23.15 (18.4–29.3)	24.3 (18.2–33.5)	24.4 (19.5–33.2)	23.95 (16.0–33.2)	0.25*
M/F (n)$^{\zeta}$	19/31	25/25	26/24	15/25	0.34**
DMFT	3.50 (0.0–8.0)***	4.0 (0.0–14.0)***	6.0 (2.0–13.0)***	7.0 (1.0–13.0)***	$p < 0.001^{*}$
PD (mm)	2.40 (1.76–2.99)***	2.21 (1.53–2.99)***	3.62 (2.56–5.65)***	3.79 (2.47–6.44)***	$p < 0.001^{*}$
PI	1.0 (0.14–1.40)***	1.73 (0.54–2.92)***	2.14 (1.0–2.92)***	2.44 (1.01–3.0)***	$p < 0.001^{*}$
GI	0.71 (0.1–1.07)***	1.0 (0.08–2.0)***	1.76 (1.0–2.0)***	1.79 (1.02–2.35)***	$p < 0.001^{*}$
CAL (mm)	0.0 (0.0–0.0)***	0.0 (0.0–0.0)***	2.62 (0.23–5.91)***	2.54 (0.39–3.98)***	$p < 0.001^{*}$
SFR (ml/min)	0.36 (0.20–0.55)	0.42 (0.20–0.55)	0.37 (0.20–0.55)	0.39 (0.20–0.60)	$p > 0.05$
Amalgam restoration	1.00 (0.00–4.00)***	1.00 (0.00–3.00)***	1.00 (0.00–5.00)***	0.00 (0.00–2.00)***	$p < 0.001^{*}$
Composite restoration	2.00 (0.00–6.00)***	2.00 (0.00–8.00)***	1.00 (0.00–5.00)***	2.00 (0.00–5.00)***	$p < 0.001^{*}$
Fixed prosthesis	0.00 (0.00–0.00)***	0.00 (0.00–0.00)***	0.00 (0.00–3.00)***	0.00 (0.00–1.00)***	$p < 0.001^{*}$

*Determined by Kruskal-Wallis test. Medians that have no superscript in common are significantly different from each other (Dunn test, $p < 0.05$)

**Determined by chi-square test

***Medians that have no superscript letters in common are significantly different from each other

$^{\zeta}$ Count for M: male, F: female

BMI boddy mass index, *M* male, *F* female, *DMFT* decayed, missing, filled teeth, *PD* pocket depth, *PI* plaque index, *GI* gingival index, *CAL* clinical attachment level, *SFR* salivary flow rate

Table 2 Salivary trace elements levels in groups Values are median (min.–max.)

Elements (ppm)	Groups				
	H (n = 50)	G (n = 50)	CP (n = 50)	GAP (n = 40)	p^*
Na	151.1 (56.5–534.6)**	205.6 (4.7–636.1)**	336.6 (112.6–1006.0)**	348.0 (118.3–695.6)**	$p < 0.001$
Mg	6.21 (2.7–17.8)**	8.46 (2.5–105.2)**	11.79 (4.8–30.8)**	11.79 (3.7–25.2)**	$p < 0.001$
K	961.3 (478.7–1011)**	1107 (674–10310)**	1441 (592.4–18320)**	1409 (447.7–15030)**	$p < 0.001$
Ca	36.53 (17.5–104.6)**	41.41 (2.4–101.1)**	61.20 (35.9–159.3)**	55.07 (38.7–104.3)**	$p < 0.001$
V	0.018 (0.009–0.032)**	0.016 (0.007–0.040)**	0.021 (0.0098–0.043)**	0.016 (0.0099–0.030)**	$p < 0.001$
Cr	0.062 (0.029–0.285)**	0.065 (0.035–0.262)**	0.089 (0.054–0.206)**	0.078 (0.055–0.141) **	$p < 0.001$
Mn	0.074 (0.028–0.612)**	0.096 (0.028–0.106)**	0.138 (0.058–1.582)**	0.088 (0.027–0.496)**	$p < 0.001$
Fe	0.908 (0.152–16.88)**	0.899 (0.25–8.597)**	2.068 (0.58–54.31)**	1.23 (0.56–11.77)**	$p < 0.001$
Zn	0.272 (0.027–8.868)	0.392 (0.004–114.2)	0.352 (0.00–13.85)	0.235 (0.00–3.60)	$p > 0.05$
Rb	0.714 (0.415–1.295)**	0.840 (0.411–1.357)**	0.979 (0.457–1.790)**	0.905 (0.340–1.576)**	$p < 0.001$
Sr	0.042 (0.006–2.674)**	0.027 (0.0008–3.195)**	0.047 (0.014–31.87)**	0.044 (0.011–0.649)**	$p < 0.001$
Se	0.012 (0.00–0.11)**	0.009 (0.00–0.062)**	0.045 (0.023–0.121)**	0.041 (0.023–0.132)**	$p < 0.001$

*Determined by Kruskal-Wallis test. Medians that have no superscript in common are significantly different from each other (Dunn test, $p < 0.05$)

**Medians that have no superscript letters in common are significantly different from each other

concentrations of Na, Mg, K, Ca, Cr, Fe, Rb, and Se were higher in the CP and GAP groups than the H and G groups ($p < 0.01$). Only the CP group exhibited significantly elevated V and Mn concentrations and this group had a significantly higher Fe concentration than the GAP group. Only Mg and K concentration were elevated in the G group when compared to the H group.

Discussion

Periodontal disease is the result of a chronic inflammatory condition that leads to oxidative destruction of supporting tissues of dentition [24]. Mineral elements are significant participants in the immunoinflammatory process and in the modulation of oxidative metabolism that occurs in the response to the microbiological challenge that occurs with periodontitis [25]. Thus, saliva would contain minerals contributed by biological entities involved in inflammation and oxidative metabolism [26]. However, other factors such as the body status of these minerals could affect the evolution and severity of the inflammatory and oxidative stress and thus the salivary content contributed by the biological entities [27]. In addition, the breakdown of supporting tissue as periodontitis progresses could be a major contributor to salivary mineral concentrations [28]. As a result, concentrations of minerals affected by periodontal diseases would be quite variable but still would be indicative of the progress and severity of the disease and with the effectiveness of any treatment [29]. Among the mineral elements that would be altered by periodontal disease are Ca, Mg, Fe, and Se, which were found to be altered with the severity of periodontal disease in the present study.

Increased extracellular Ca2+ could increase intracellular calcium [30]. Increased intracellular Ca2+ is considered a signal to initiate the inflammatory process [31]. Also the increased intracellular Ca^{2+} induces the production of cytokines such as tumor necrosis factor-alpha (TNF-α), which are priming agents that stimulate phagocytic cells to produce reactive oxygen species [32, 33]. The reactive oxygen species are used to destroy offending materials such as bacteria [34]. If the production of reactive oxygen species is excessive or not adequately controlled, they result in pathological conditions such as breakdown of tissue that occurs with periodontal disease [35]. The increase intracellular Ca and the breakdown of calcified tissue with periodontal disease should result in an elevation in saliva Ca. In addition, matrix metalloproteinases (MMP) are Ca-dependent enzymes that are involved in the degrading of the extracellular matrix of periodontal tissues.

Increased MMP1 and MMP8 have been found in gingival crevicular fluid and saliva during periodontitis [36, 37]. This increase in MMPs could also contribute to an increased salivary Ca. Ashley et al. [38] found a statistically significant relationship between the concentrations of Ca in saliva and plaque. Consistent with this finding, we found significantly higher salivary Ca concentrations in the CP and GAP groups, which had a higher PI. Thus, diffusion gradient between dental plaque and saliva can also contribute to an elevated concentration of salivary calcium [38, 39]. The preceding provides a basis for the finding in our study and by others [40–42] of elevated concentrations of salivary Ca in patients with periodontitis in comparison to healthy control groups. Also, our finding that the CP and GAP groups with more severe clinical periodontal pathology had a significantly higher salivary Ca, in comparison to the H and G groups support the conclusion of others that increased plaque and increased bone loss are the reasons for elevated Ca in saliva with chronic or aggressive periodontitis [43, 44]. Also, increased salivary Ca influences the mineralization of plaque and causes calculus formation in periodontitis patients [45]. Our findings indicate that salivary Ca concentration could be used as an indicator of severe periodontitis or the effectiveness of its treatment.

Magnesium has been called nature's physiologic Ca^{2+} channel blocker. Thus, Mg can affect inflammatory and oxidative stress thorough altering the role of Ca^{2+} in these processes. For example, in Mg deficiency, cellular Ca^{2+} increases through an influx from extracellular sources via slow Ca^{2+} transport channels. In addition, Mg deficiency increases the efflux of K^{+} from cells via Mg^{2+}-sensitive K^{+} channels [46]. Magnesium also has anti-oxidant activity through the reduction of peroxide radicals [47]. Thus, Mg status could have a marled effect on the progress and severity of periodontal disease. A low Mg status, which apparently occurs quite often [48], could add to the chronic inflammatory and oxidative stress in periodontal disease. However, a deficient Mg status would not necessarily result in a decreased salivary Mg concentration. Magnesium occurs in a high amount in calcified tissue and thus its breakdown could result in a paradoxical increase in saliva Mg concentration although overall body status is low. In our study, individuals in the G group, with the least clinical periodontal measurements of periodontitis exhibited significant increases in only Mg and K in saliva. The increase in Mg may be an indication that inflammatory or oxidative stress is starting. The formation of reactive oxygen species might have stimulated an increase in oral fluid Mg to fulfill its anti-oxidant function or to moderate the Ca^{2+} priming effect in the inflammatory response [49, 50]. However, the further elevation in salivary Mg in the CP and GAP groups suggests that the control has not prevented oxidative destruction of calcified tissue, which is a major site of the body's Mg. Shetty TJ et al. [49] also found higher mean salivary Mg concentrations in patients with gingivitis (0.166 ± 0.028 ppm) and periodontitis (0.205 ± 0.066 ppm) when compared to healthy individuals (0.135 ± 0.028 ppm). Although these results are consistent with the results of our study, patients' salivary Mg concentrations in our study much higher than in Shetty TJ's study. This can be attributed to different methods used for element analysis or may be related to the patient population with different severity of inflammation. Manea and Nechifor [50] reported a non-significant increase in salivary Mg concentrations in patients with periodontitis. These findings suggest that an elevated salivary Mg concentration, perhaps above 8 ppm, could be used to support the diagnosis of periodontal disease. In addition, changes in salivary Mg concentration may help in determining the progress of periodontal disease and any treatment of it.

As indicated above, Mg also affects the movement of K in and out of cells. Thus, Mg status of the patient and Mg changes caused by the inflammatory response likely affected the concentration of K in gingival fluid. This suggestion is supported by the finding that K was the only other significant increase in the G group compared to the H group. Like Mg, the K concentration in saliva was further increased in the CP and GAP groups. Thus, K changes with periodontal disease apparently mirror those of Mg. Like Mg, the K increase in severe periodontal disease likely arises from degenerated epithelium and connective tissue [51–53]. This was also suggested by Kaslick et al. [54] who also found increased salivary K concentrations in periodontal disease. Thus, the combination of salivary K and Mg concentrations could be used as in the diagnosis of active disease in periodontal tissue and reflect its clinical status.

Aun et al. [55] found that periodontitis is associated with higher Na salivary concentrations than those with periodontal health, which they suggested may have been caused by the sudden change in the amount of Na introduced into the extracellular space and alveolar bone destruction caused by periodontal disease. Others have reported increased Na concentrations in the extracellular matrix and saliva [54–56] and have suggested that his occurs through cellular exchange to gingival fluid and the degeneration of epithelium and connective tissue in severe inflammation [56]. Thus, increased salivary Na might be used as a marker for chronic and aggressive periodontal disease and may reflect the clinical status of periodontal tissues.

Selenium is a component of enzymes involved in anti-oxidant mechanisms needed to counter oxidative stress. Salivary Se concentrations were higher in individuals with chronic and aggressive periodontitis compared to healthy and gingivitis individuals. This may be attributed to an increased need for Se for enzymes, such as glutathione peroxidase involved in anti-oxidant action against the excessive reactive oxygen species that occurs with severe periodontal disease [57]. This suggestion is supported by the finding that the inflammatory process triggers the gingival anti-oxidant defense mechanism which increases selenium-containing glutathione peroxidase in biologic fluids [58]. Thus, an elevated

selenium concentration in saliva could be used to support clinical measures to assess the severity of periodontitis and the progress of treatment of the disease.

Iron has roles that can affect its content in saliva. Iron participates in the Fenton reaction that creates reactive oxygen. Iron also is essential element for the growth of anaerobic microorganisms in cells, tissues, and biological fluids [59, 60]. Both roles could increase salivary Fe content, which was found in the CP and GAP groups in the present study. Groenink et al. [61] found higher Fe concentrations in gingival crevicular fluid (GCF) in patients with periodontitis than in periodontally healthy patients. They suggested that the increased iron concentrations were caused by increased hemoglobin concentration with inflamed periodontal tissue and/or subgingival dental plaque microorganisms [61, 62]. This suggestion was supported by an animal study showing that iron may play an important role in active periodontal disease [63]. The significant difference in the extent of the increase in the iron concentration between the CP and GAP groups is difficult to explain. Perhaps this reflects a difference in the inflammatory process increasing hepcidin synthesis that decreases recycling of Fe from macrophages.

Differences in the elevation in salivary Fe may be useful in determining the stage of severe periodontal disease. However, because salivary iron concentrations may be affected by gingival bleeding caused by gingival inflammation [64], further studies ascertain whether blood contamination correlates with saliva iron concentration [65].

There was no statistically significant difference among the groups in salivary Zn concentration. This finding was consistent with Kuraner et al. [40] who found no difference in salivary Zn concentration between healthy individuals and those with periodontitis. This is surprising because Zn, which is found in the saliva, plaque, and pellicle in different concentrations, limits acid production in dental plaque and inhibits the colonization of bacterial pathogens [66–68]. In addition, Zn is required for immune regulation and is a cofactor for superoxide dismutase (SOD), an important anti-oxidant factor. [69] This suggests that Zn would increase in saliva with periodontal disease. Perhaps, Zn status has an inhibitory effect on increasing the zinc content of saliva. It has been reported that serum Zn concentrations are lower in individuals with periodontitis than healthy individuals [70, 71]. This could be the result of deficient intakes or because serum zinc concentrations fall with inflammation. Salivary Zn does not appear to be useful for the determination of periodontitis or its treatment.

Salivary Sr, V, Mn, Rb, and Cr levels were higher in individuals with chronic periodontitis compared to the other groups. It is thought that these elements are higher in periodontitis groups because of releasing from dental restorations in mouth [72, 73] or increased amount of calculus. [74]. These mineral elements also might be increased through bone breakdown because bone is a major site of Sr, V, Mn, and Rb [75, 76]. Although measurements of these minerals in saliva could add to assessment of the severity or treatment of periodontitis, they would only be additive to the more important increases in Mg, Ca, K, Na, Se, and Fe measurements.

This study had several limitations. First, instead of the classification of periodontal diseases newly described by European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP) [77], we used 1999 international classification criteria [21] because we started and collected saliva samples between 2014 and 2016. If the groups are classified according to the new criteria, the intergroup salivary macro and trace elements levels might vary. The other limitation of the study is the elemental release may have been related with the dietary intake level. Also because reports indicating the threshold values of salivary mineral element concentrations for the diagnosis of periodontal disease are quite limited, in our cross-sectional study, conclusions were based on the comparisons between the salivary element concentrations of individuals with different periodontal conditions. Therefore, further studies are needed to understand the significance of changed salivary mineral element concentrations in the pathogenesis of periodontal disease.

Conclusion

Although salivary analysis cannot fully replace the gold standard examinations of periodontitis performed by the dentists, it can be convenient and noninvasive tool for diagnosis and prognosis, and for treatment monitoring. Our study indicates that the measurement of Mg, Ca, K, Na, Se, and Fe could help in assessing the onset and severity of periodontal disease and the progress of any treatment. However, the large variation in the concentrations of the elements in each of the various stages of periodontal disease and the lack of a reference values for the minerals in saliva of healthy individuals has a limiting effect for the diagnosis periodontitis. Some of the variation in the measurements probably were caused by exogenous factors such as disease and nutritional status. The present study indicates, however, that assessing the change in these minerals would be useful in assessing the progress of any treatment periodontal disease.

Funding Information This study was supported by a grant from the Scientific Research Projects of Selcuk University (SELCUK/BAP-14102025).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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