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Original Article

Induces of periodontitis increases salivary orosomucoid levels

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Abstract

Aim: Periodontitis is characterized by chronic infection and inflammation in periodontal tissues leading to destruction of dental support tissues. Acute-phase proteins are effective markers for the identification and evaluation of inflammatory diseases including periodontitis. Orosomucoid (ORM) is an acute-phase plasma protein, also called alpha-1-acid glycoprotein, which is inflammation sensitive. The aim of this study was to evaluate and compare the salivary levels of ORM and C-reactive protein (CRP) in individuals with/withoutperiodontal disease.

Material and Methods: A total of 90 subjects were divided into two groups of 45 patients each; periodontally healthy and chronic periodontitis. Saliva samples were collected, and clinical periodontal parameters and salivary flow rates were evaluated at baseline. The ORM and CRP levels in the saliva were analyzed with enzyme-linked immunosorbent assay.

Results: The periodontal clinical parameters were significantly higher in the chronic periodontitis group when compared to those of the healthy controls ($P<0.05$). There was no significant difference in the salivary flow rate between the chronic periodontitis and periodontally healthy groups ($P>0.05$). However, the CRP and ORM levels in the saliva were significantly higher in the chronic periodontitis patients than in the healthy controls ($P<0.05$). In addition, a significant positive correlation was found between the CRP and ORM levels in saliva of all groups ($P<0.05$).

Conclusions: This study revealed that presence of periodontitis was associated with higher salivary CRP and ORM levels. Moreover, the ORM level seemed to be associated with tissue destruction in inflammatory periodontal disease.

Keywords: Acute-Phase Protein; Orosomucoid; Periodontitis; Saliva.

INTRODUCTION

Periodontitis is a complex chronic infectious disease affecting and destroying the tooth-supporting periodontal tissues (1). Chronic periodontitis is one of the most common chronic inflammatory diseases, affecting 20–50% of the adult population worldwide (2). The host's inflammatory mediators cause reabsorption of the alveolar bone which is supporting the teeth, and detachment of the connective tissue from the root surface (1). Acute-phase proteins, cytokines, and prostaglandins are mediators that are part of the host response causing tissue destruction (3).

The release of acute-phase proteins into the circulatory system is called an "acute phase response" and constitutes the initial reaction against a bacterial infection (3). The acute phase reaction is a non-specific body response in the initial stages of infection, injuries, ischemic necrosis, or malignancy (4). Acute phase proteins are also part of the innate immunity, not only in acute inflammation, but also in long-standing chronic conditions (5). It has been suggested that acute phase proteins can be sensitive markers for evaluating the inflammatory conditions of various microbial infections, including periodontitis (3). The C-reactive protein (CRP) is an acute phase protein that is a sensitive marker for evaluating the systemic inflammatory status (6). Shojaee et al. (7) showed that the salivary CRP levels in chronic periodontitis patients are higher than those in periodontally healthy individuals. Moreover, the authors suggested that there was a significant relationship between periodontitis and the salivary CRP concentrations (7).

Orosomucoid (ORM), also called alpha-1-acid glycoprotein (AAG), belongs to the family of acute phase proteins and constitutes about 1–3% of all plasma proteins (8). It is produced mainly in hepatocytes, but can also be found in

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the extrahepatic tissues, especially in white adipose tissue (9). This protein, a typical determinant of inflammation, is mainly induced by IL-1, TNF- α, and IL-6 polypeptides (9). Its function has not yet been fully elucidated, but it is a known immunomodulator. ORM inhibits both mitogen-induced lymphocyte proliferation and platelet aggregation, as well as neutrophil chemotaxis, superoxide production, and aggregation (10).

Rangé et al. (11) found that the plasma ORM concentrations in patients with severe periodontitis were higher than those in mild to moderate obese periodontitis patients. They suggested that the severity of periodontitis was associated with increased ORM levels in obese individuals (11). In addition, it was stated that only the CRP level increased in early onset inflammation reactions, then the CRP and ORM levels increased (11). After successful treatment, the CRP level decreased first (11). Since periodontitis is a chronic infectious disease, they suggested that ORM was a better inflammatory marker than CRP in obese individuals with periodontitis (11). However, it was stated that the study had certain limitations due to the cross-sectional design, so a causal relationship could not be established (11). In a recently published review, it was noted that measuring the ORM level to assess the progression of a disease may be more effective than measuring the CRP concentration alone (12).

In the literature, a few studies have revealed a relationship between ORM and periodontitis therefore the relationship between ORM and periodontal disease was not fully elucidated. The aim of this study was to determine the CRP and ORM levels in the salivary samples of chronic periodontitis patients and periodontally healthy controls, and compare these values with the clinical parameters. In addition, the present study was focused to detect the role of ORM on the presence of periodontitis via comparing with salivary levels of CRP.

MATERIAL AND METHODS

Selection of subjects

The study volunteers were selected from among those patients who presented to the Periodontology Department at the Faculty of Dentistry at Ordu University from October 2016 to February 2017 and signed informed consent forms. The study protocol was approved by the Clinical Research Ethics Committee of Ordu University in Ordu, Turkey in accordance with the Helsinki Declaration of 1975, as revised in 2008 (Protocol ID: 2016-84). The assessment of the periodontal status of the volunteers was defined according to the criteria set by the American Academy of Periodontology in 1999 (13). According to these criteria, those individuals with 30% bone loss radiologically and at least 2 or 3 teeth with pocket depths of more than 5 mm were regarded as periodontitis patients. Those individuals with pocket depths of less than 3 mm, no redness in the gingiva, and no bleeding on probing were considered to be periodontally healthy controls. This study was conducted with ninety individuals aged 25–55 years old who met these criteria. The individuals were classified into two groups: a periodontally healthy control group (n=45; 24 males and 21 females; age: 38.30±3.80 years) and a chronic periodontitis group (n=45; 24 males and 21 females; age: 40.2±3.15).

Inclusion Criteria

Inclusion criteria for the patients were as follows: 1) never-smokers; 2) no history of systemic disease; 3) no patients had been under periodontal treatment and medicine for at least 6 months before the study; 4) no pregnancy or lactation; 5) no aggressive periodontitis, no periapical pathologies, no excessive forces including mechanical forces caused by orthodontic forces and occlusal forces 6) no allergy or sensitivity to any drug, 7) possess ≥20 teeth excluding third molars, and teeth with advanced decay 8) GI = 0, PD ≤ 3 mm, and no signs of bone loss by clinical and radiographic examination for the periodontally healthy groups; and 9) clinical signs of inflammation (red color and swelling of the gingival margin) GI ≥ 2, PD and CAL ≥ 5 mm, and bone loss affecting > 30% of the existing teeth on clinical and radiographic examination for the generalized chronic periodontitis groups.

Periodontal examination

In all of the patients, the following indexes were used routinely for the periodontal examination: the Silness-Löe plaque index (PI) (14) to measure the plaque formation and accumulation on the teeth, the Löe-Silness gingival index (GI) (15) for the diagnosis of gingival inflammation, the probing pocket depth (PPD) and clinical attachment level (CAL) to measure the degree of periodontal disease, and the bleeding on probing (BOP) (16) index to determine the periodontal disease activity. Routine radiographic evaluations were performed to detect the bone levels. The procedures were routinely performed with a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA) calibrated in millimeters. No invasive procedures were used during these examinations. The clinical parameters of the patients were measured by the same investigator (F.O.D.), who was blinded with respect to the study design.

Saliva sampling

All samples were obtained in the morning following overnight fasting, during which patients were requested not to drink (except water) or eat. Before measuring the periodontal indexes, unstimulated salivary samples were collected from the participants to determine the inflammatory cytokine levels. While seated upright in a comfortable environment, after rinsing their mouth with distilled water and spitting, they were asked to swallow the saliva in their mouth. Then, for five minutes, they were asked to spit into a Falcon tube without swallowing the saliva that accumulated in their mouth (17). The saliva flow rate was calculated in ml/min by dividing the amount of saliva collected by the time at which the saliva was collected (18). Next, 1.5 ml of the collected saliva was transferred to an Eppendorf tube. The salivary samples were immediately centrifuged to remove cellular debris (10,000 g × 10 min at 4ºC), and the supernatants (50µL each) were stored at -40oC until the analysis.

Biochemical analysis

The CRP and ORM levels in the saliva were measured via ELISA using commercially available kits (Hangzhou Eastbiopharm Company, Zhejiang, China (Mainland)). The levels of CRP were expressed as ng\ml, while the levels of ORM were expressed as mg\l.

Statistical analysis

The primary outcome variable (salivary CRP level) was used to determine the sample size calculation. For this purpose, the power value for obtaining the effect size was 0.573 (alpha=0.05). For the CRP parameter measured in this study, 45 patients in each group was equal to 84%.

The Shapiro-Wilk test was used to determine whether the data were normally distributed. The comparisons of the biochemical and clinical parameters were analyzed using the Mann-Whitney U non-parametric test, after the normality of the data failed.

Spearman's rank correlation test was used to detect the relationships between the CRP and ORM levels and the periodontal clinical parameters. All of the tests were performed using statistical software (SPSS version 20.0; SPSS Inc., Chicago, IL, USA). P<0.05 was considered to be statistically significant.

RESULTS

The clinical parameters of the chronic periodontitis and periodontally healthy groups are outlined in Table 1.

There were no significant differences in the age and gender proportions among the individuals in both groups (P>0.05). The PI, GI, PPD, CAL, and BOP were significantly higher in the chronic periodontitis group than in the control group (P<0.001). However, the salivary flow rates were not significantly different between the chronic periodontitis and periodontally healthy groups (P=0.187). The salivary CRP and ORM levels were significantly higher in the chronic periodontitis group than in the control group (P=0.006 and P=0.033, respectively) (Table 2).

Moreover, the salivary CRP levels were significantly correlated with the ORM levels in both groups (r=0.689, P=0.000). In addition, the PI, GI, and BOP values were positively correlated with the salivary ORM and CRP levels in both groups (P<0.05) (Table 3).

Table 1. Clinical parameters of groups with chronic periodontitis and periodontally healthy control

Groups		N	Mean	Std. Deviation	Median	Min	Max	IQR	P-values
SFR	CP	45	0.77	0.48	0.48	0.20	2.30	0.40	0.187
	Control	45	0.69	0.42	0.42	0.30	1.90	0.35	
PI	CP	45	1.65	0.42	0.42	0.90	2.43	0.67	<0.001
	Control	45	0.51	0.32	0.32	0.00	1.08	0.52	
GI	CP	45	1.62	0.36	0.36	0.83	2.24	0.54	<0.001
	Control	45	0.35	0.25	0.25	0.00	0.79	0.47	
PPD (mm)	CP	45	2.11	0.51	0.51	1.43	3.44	0.81	<0.001
	Control	45	1.50	0.13	0.13	1.37	1.78	0.19	
	CP	45	2.73	0.64	0.64	1.61	4.18	0.83	<0.001
	Control	45	1.50	0.13	0.13	1.37	1.78	0.19	
BOP (%)	CP	45	65.38	15.98	15.98	34.57	90.35	26.28	<0.001
	Control	45	0.00	0.00	0.00	0.00	0.00	0.00	

Significant difference between groups, P<0.05

Table 2. Salivary Orosomucoid (ORM) and C-reactive protein (CRP) levels of groups with both chronic periodontitis and periodontally healthy control

Groups		N	Mean	Std. Deviation	Median	Min	Max	IQR	P-values
ORM (ng/ml)	CP	45	1.49	0.61	1.27	0.83	3.56	0.43	0.033
	Control	45	1.34	0.91	1.18	0.63	2.50	0.30	
CRP (mg/L)	CP	45	0.85	0.29	0.84	0.41	1.58	0.31	0.006
	Control	45	0.70	0.23	0.70	0.32	1.44	0.17	

Significant difference between groups, P<0.05

Table 3. The Spearman's rank correlation (rho) among all groups with levels of ORM and CRP, PI, GI, PPD, CAL, and SFR

		SFR (ml\dk)	PI	GI	PPD (mm)	CAL (mm)	Salivary ORM	Salivary CRP
PI	r	.098						
	p	.357						
GI	r	.071	.906**					
	p	.508	.000					
PPD (mm)	r	.068	.668**	.777**				
	p	.523	.000	.000				
CAL								
(mm)	r	.066	.796**	.860**	.883**			
	p	.534	.000	.000	.000			
Salivary								
ORM	r	-.043	.242*	.241*	.155	.187		
	p	.687	.021	.022	.145	.078		
Salivary								
CRP	r	.106	.346**	.273**	.179	.211*	.689**	
	p	.319	.001	.009	.092	.046	.000	
BOP								
(%)	r	.094	.885**	.914**	.777**	.871**	.250*	.296**
	p	.380	.000	.000	.000	.000	.018	.005

**:Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

DISCUSSION

In the current cross-sectional study, the CRP and ORM levels were compared by taking salivary samples from generalized chronic periodontitis and periodontally healthy individuals. The analysis of the data collected from this research supports a significant relationship among CRP, ORM, and chronic periodontitis which is consistent with the results of a previous comparative study (19).

Diagnostic salivary tests hold promise for the future due to their ease of use, without the need for special equipment and personnel, non-invasiveness, painlessness, and high repeatability (20). The representation of all of the periodontal regions provides a comprehensive assessment of the disease state, as opposed to site-specific gingival crevicular fluid (GCF) (21,22). The saliva includes tissue metabolites, GCF, and immunological structures (18,23), and reflects the predominant intra-oral condition (24). Salivary diagnostic tests can help to visualize large populations, and in this context, tests with high sensitivity and high specificity are valuable (24). Stimulation can increase the flow of GCF, which can lead to erroneous concentrations of certain substances in the saliva (22). Therefore, in this study, all of the samples were collected without citric acid, gum, or paraffin stimulation.

Shaila et al. (25) showed that SFRs were not significantly different among gingivitis patients, periodontitis patients, and healthy subjects. Similarly, the results demonstrated that there was no significant association in the SFR values between the periodontitis and control groups. In addition, no significant correlation was found between the SFR and clinical parameters in all groups. Based on these findings, the present authors suggested that SFR may not be directly related to the presence of periodontal disease.

Several studies have demonstrated that there is a positive correlation between the presence of chronic periodontitis and elevated serum CRP levels, because biologically, inflammatory mediators (IL-1, IL-6, and TNF-α) are released under periodontitis conditions and stimulate the hepatocytes to produce CRP (3, 26-28). A meta-analysis of CRP in relation to periodontitis showed strong evidence that the plasma CRP level in cases of periodontitis was high when compared to the controls (5). Recent studies have found that serum CRP levels are higher in periodontitis patients compared to control subjects (3, 28), whereas there is no statistically significant difference in this level (6). On the other than, a study showed that saliva CRP levels increased in patients with chronic periodontitis (7) when compared to the control group, while another study found no difference in this level between the both groups (29). Different outcomes may be affected by the complexity of periodontitis progression and\or multifactorial factors. Similar to Shooje et al. (7) study, the present study showed that levels of the saliva CRP were significantly higher in the CP group than control group. Our data may reveal the importance of CRP analysis in the saliva in the determination of periodontal inflammation. Saliva samples in the periodontitis diagnosis is more cost-efficient, simple, rapid, non-invasive and more acceptable to the individual when compared to serum samples.

It is believed that the ORM is a true independent marker in the detection of disease activity, and may have clinical effects on the diagnosis of the inflammatory condition of periodontal disease (11). The ORM levels have been assessed for their immunomodulator potential due to their association with lymphocytes and proinflammatory cytokines (30). This protein is a typical marker of inflammation, and increases 3–4 folds after an inflammatory stimulus (31,32). Neutrophils and monocytes can also synthesize ORM, thus contributing to the serum level of this protein in the case of sepsis (8). Interestingly, CRP is an acute phase inflammatory marker, while ORM is a chronic phase marker (33, 34). Since the ORM level is believed to be sensitive and specific for the diagnosis and possible prognosis of chronic disease, it is seen as a reliable biomarker in the diagnosis of periodontal disease.

Rangé et al. (11) suggested that the ORM level was associated with the severity of periodontal inflammation, after adjusting for age, sex, and smoking. In a comparative study of systemic inflammation in cardiovascular disease (CVD) and periodontal disease, when examining venous blood serum samples, it was reported that the ORM and CRP levels of individuals with both diseases were higher than those in healthy individuals and those in individuals with only CVD or periodontitis (19). An increased ORM level is a characteristic feature of individuals who have both CVD and periodontitis (11).

Pinho et al. (35) found that elevation blood levels of CRP and AAG in patients with periodontal disease were reduced after non-surgical periodontal treatment. Also, when the controls and periodontal disease individuals were compared, it was determined that blood CRP and AAG levels were higher in patients with periodontal disease, and significantly higher despite the decrease in these levels 3 months after periodontal treatment (35). After 6 months the AAG levels for periodontal disease group were higher when compared to the control group, while there were no statistically differences in the CRP levels (35). In addition, authors showed that there was a positive correlation between BOP values and AAG levels at the 0-3 month's examinations (35).

To the best of our knowledge, this is the first study to investigate the saliva levels of ORM. Our data demonstrated that saliva levels of ORM were higher in CP group than the control group. We also found positive correlations among salivary ORM, CRP levels and PI, GI, BOP values in all groups. Based on these findings, the present authors suggested that ORM has been detected to display properties of an acute-phase protein in the saliva of subjects with periodontal health and disease. Also, the increase in levels of this protein in the saliva can reveal the presence of periodontal disease.

The present study did have some limitations. For example, since the study was cross-sectional, it was not possible to determine the causality of the relationships. We do not know whether periodontitis increases the CRP and ORM levels. In addition, CRP and ORM are not specific to periodontitis; these proteins are indications of a wide spectrum of diseases, such as trauma, infection, and inflammation. To minimize these variations, we included only those participants who did not have systemic disease, did not smoke, and did not take any medication in the previous six months (36).

CONCLUSIONS

Within the limits of this study, it can be suggested that the salivary CRP and ORM are related to periodontal sease. The salivary ORM concentrations may be useful for the detection of periodontal disease. However, further long-term studies, eliminating the limitations, are required to explain the role of ORM in the pathogenesis of periodontal disease, and to confirm these findings.

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