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Comparative evaluation of the cortisol level of unstimulated saliva in patients with and without chronic periodontitis

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ABSTRACT

Background: Chronic periodontitis (CP) is one of the most prevalent diseases of the oral cavity with various biological and behavioral risk factors. We aimed to evaluate the association between the salivary cortisol level (SCL) of unstimulated saliva and CP in patients referred to Isfahan Dental Faculty.

Materials and Methods: In this analytic cross-sectional study, 90 patients were selected based on the presence of periodontitis and were divided into two groups: with periodontitis and without periodontitis (n = 45). First, by evaluating the level of anxiety with Spielberger State-Trait Anxiety Inventory questionnaire, each group was divided into three subgroups, each containing 15 persons. To measure the SCL in all subgroups by the enzyme-linked immunosorbent assay method, saliva samples were collected with unstimulated spitting method between 9 and 11 AM. Periodontal evaluation was done using the mean probing depth (PD), plaque index, and bleeding on probing. The obtained data were analyzed using SPSS software (version 20, IBM Corp., Armonk, N.Y., USA) and analysis of variance, independent t-test, Chi-square, Mann–Whitney, Spearman correlation, and Pearson correlation coefficient tests (α = 0.05).

Results: The mean level of salivary cortisol (P = 0.048) and PD (P = 0.009) in patients with periodontitis was significantly higher than those without periodontitis. There was a direct and meaningful correlation between PD and SCL (P < 0.001, r = 0.363). In both groups of participants with (P < 0.001) and without periodontitis (P < 0.001), the mean SCL in patients with high anxiety was significantly more than patients with medium and low anxiety.

Conclusion: Our results showed that there is an increased level of salivary cortisol (as anxiety index) in patients with CP. Therefore, it seems that the probability of the occurrence of periodontitis is higher in those with increased cortisol level.

Key Words: Anxiety, chronic periodontitis, glucocorticoid, periodontal index, saliva

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INTRODUCTION

Periodontitis is the periodontal inflammatory response that leads to the destruction of the tooth-supporting tissues. It is one of the most prevalent causes of tooth loss, although the presence of periopathogenic bacteria is essential to initiate the process and their mere presence is not sufficient.[1,2] The importance of biological and behavioral risk factors for periodontal

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diseases, including smoking, poor oral hygiene, and systemic diseases such as diabetes mellitus, has been proven.[2,3] Psychological stress is still not known as an absolute risk factor in the development of periodontal disease.[4,5] However, it can influence cellular immunity by suppressing the function of natural killer cells and lymphocytes and inhibit their mitosis.[6] Similar to other chronic diseases, the development of periodontitis may be associated with conditions that affect host resistance to periopathogenic bacteria. Evidence suggests that progression of chronic periodontitis (CP) may be affected positively by chronic stress, depression, and anxiety.[7,8]

Cortisol is a glucocorticoid hormone, which is released by adrenal glands and has various effects on the body's physiological structures. Any type of stress (physiological or neurological) can immediately and significantly increase the release of adrenocorticotropic hormone from the anterior pituitary gland, followed by increased secretion of cortisol from the adrenal cortex within a few minutes. In the case of chronic stress, cortisol remains persistently elevated.[9] Glucocorticosteroid increase during a long period suppresses immunity by reducing the secretion of immunoglobulin A (IgA) (effective in inhibiting colonization of periodontal pathogens), IgG (opsonization), and neutrophil function.[10,11]

Salivary cortisol is indexed under free serum cortisol or biological active cortisol and is not under the influence of protein-bound cortisol.[12,13] Due to the high correlation between salivary cortisol level (SCL) and unbound cortisol level in the serum,[14] SCL is a biomarker normally used to study the function of the hypothalamic–pituitary–adrenal cortex (HPA) during stress.[15,16]

Refulio *et al*.[17] indicated that patients with moderate and severe CP had significantly higher SCL than patients with mild CP and those with high SCL, and depression may have an increased risk for CP. Mannem and Chava[18] concluded that CP showed a significant correlation with hypercortisolemia, work tension, economic problems, clinical stress syndrome, plaque index (PI), and unsecured job. In contrast, Mengel *et al*.[19] did not find a relationship between factors in peripheral blood, stress, and periodontal disease. Johannsen *et al*.[20] studied the amount of interleukin-1ß (IL-1ß), IL-6, matrix metalloproteinase-9, and cortisol of gingival crevicular fluid (GCF) in women with stress-related depression. The results of this study showed that plaque accumulation and gingival inflammation were higher in the case group and IL-6 and cortisol levels of GCF had increased compared with the control group. However, the significant correlation between cortisol levels in the saliva and periodontal disease could not be determined. According to controversial studies, the importance of effective factors in periodontal disease, and few human studies about the relationship between SCL and CP, we aimed to investigate the association between CP with anxiety and SCL in patients referred to Isfahan Dental Faculty.

MATERIALS AND METHODS

This study has been approved by resaerch and ethics committe of isfahan university of medical sciences, dental school (No:395311). In this analytic cross-sectional study, 90 patients aged 20–55 years who referred to the Department of Periodontology, School of Dentistry, Isfahan University of Medical Sciences (2015–2016), were selected randomly and divided into two groups (n = 45) based on the presence or absence of CP: With CP (cases) and without CP (control). Patients had at least 15 natural teeth except for the third molars did not eat or drink for at least 1 h before the test.

Exclusion criteria

Exclusion criteria included:

1. Patients who had systemic conditions that affect periodontal disease,
2. Chronic use of corticosteroids or immunosuppressive drugs as well as having immunosuppressive diseases,
3. Antibiotic intake during the past 6 months or at the time of study,
4. Periodontal treatment in the past 6 months,
5. Smoking and alcohol consumption,
6. Pregnancy and lactation,
7. Having symptoms of acute illness or pulpal pain at the time of the study,
8. Being under treatment for stress,
9. Taking medicines which affect salivary glands such as antihistamines and tricyclic antidepressants,
10. Antihypertensive, diuretics or psychotropic drugs and
11. Those undergoing orthodontic treatment.

Definition of periodontitis was based on the classification of the American Academy of Periodontology (AAP): having at least two areas between the teeth with ≥4 mm attachment loss

or pocket depth of ≥5 mm at interdental areas in two different teeth.[21] The severity of periodontal disease was classified on the basis of AAP and the amount of clinical attachment loss (CAL), as follows: mild (1–2 mm), moderate (3–4 mm), and severe (≥5 mm).[21]

Study conditions were explained to patients, and after signing a consent form, they voluntarily entered the study. Demographic variables, including age, sex, occupation, the level of education, and frequency of daily brushing, were recorded. Then, anxiety level was measured through responses to the Spielberger State-Trait Anxiety Inventory (STAI) questionnaire.[22] This self-report questionnaire measures two domains: state anxiety and trait anxiety. State anxiety consists of twenty statements that evaluate how respondents feel "right now, at this moment." Trait anxiety consists of twenty statements that evaluate how respondents feel "generally" by choosing one of four options (1 = almost never, 2 = sometimes, 3 = often, and 4 = almost always). We used the trait anxiety part to evaluate general anxiety. A score of 1–4 was given to each item (higher scores suggesting greater levels of anxiety), and the response scale indicates frequency (4 indicates the presence of high anxiety). Scoring for the 11 negatively worded items that show the presence of anxiety was based on the order of the test form, and for the 9 positively worded items that do not show expression of anxiety, it was reversed. Total scores ranged from 20 to 80. According to the classification of the questionnaire, we divided participants into the following three groups, in order to interpret the results easier: (1) low anxiety level (≤31), (2) moderate anxiety level (32–52), and (3) high anxiety level (≥53).

It should be mentioned that if the number of each group based on anxiety (low, moderate, and severe) had not reached 15 participants, we would have added new samples with periodontitis (based on its definition) in the case group or without periodontitis in the control group.

The participants were examined by a dental student under the supervision of a periodontist. The following periodontal indices were evaluated using a Williams probe (Hu-Friedy, Chicago, IL USA): the mean probing depth (PD) was recorded in millimeters by measuring the distance from gingival margin to the bottom of pocket at 4 areas around each tooth (mid-buccal, mid-lingual, mesial and distal).[23] Silness and Loe PI was calculated using a standard probe and direct observation (naked eye), and the amount of plaque was scored at four levels: 0 – no plaque, 1 – a film of plaque adhering to the free gingival margin and adjacent area of the tooth which may be seen using the probe on the tooth surface, 2 – moderate accumulation of soft deposits within the gingival pocket or the tooth and gingival margin which may be seen with the naked eye, and 3 – abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin. An average score was calculated for the whole mouth.[24] Bleeding on probing (BOP) was assessed based on Ainamo and Bay index through observation of the presence or absence of bleeding 30–60 s after gentle movement of the probe in four areas (mid-buccal, mid-lingual, mesial, and distal) and determining the percentage of bleeding surfaces.[25] Unstimulated saliva was collected through spitting method between 9 and 11 AM to minimize the effect of circadian rhythm.[9,26,27] Patients were instructed not to brush, drink, or eat meals for 1 h before going taking the test in order to prevent salivary contamination and then were asked to sit upright for 30–60 min on the unit. Immediately, before the test, they were asked to wash their mouth with water and after that to tilt their head slowly forward and to expectorate saliva collected in their mouth within 5 min in a 15-ml disposable test tube which was equipped with funnels for ease of testing. Patients were reminded not to swallow or stimulate saliva by movements of their head during collection of saliva. All samples were stored at −20°C in the freezer. After collection of all samples, they were centrifuged at 3000 rpm by a laboratory specialist based on the instructions of the laboratory kit. SCL was measured in nanogram/milliliter (ng/ml), using the enzyme-linked immunosorbent assay method and human cortisol saliva kit (DiaMetra human cortisol saliva, Italy).

The normal range was defined between 2.5 and 10 ng/ml based on the laboratory kit. Data were analyzed by univariate analysis of variance, independent *t*-test, as well as Chi-square, Mann–Whitney, Spearman correlation, and Pearson correlation tests ($\alpha = 0.05$).

RESULTS

The study was performed on 90 patients with (case group) and without (control group) CP with an average age of 37.1 ± 9.8 years (range: 23–55 years) and 34.8 ± 10.7 years (range: 20–55 years), respectively ($P = 0.29$, independent *t*-test).

Table 1 shows the distribution of the participants with respect to sex, level of education, and frequency of daily brushing.

There was no significant difference between the two groups with respect to sex ($P = 0.245$, Chi-square test), education level ($P = 0.407$), and frequency of daily brushing ($P = 0.672$, Mann–Whitney test). Among the participants in the case group, 67.4% were housewives, 20.9% were employees, 2.3% were self-employed, and 9.3% were students. In the control group, 65.1% were housewives, 14% were employees, 9.3% were self-employed, and 11.6% were students.

Figure 1 shows the comparison results of mean SCL, PD, BOP, PI, and anxiety scores in the two groups.

T-test showed that the mean PD ($P = 0.009$) and SCL ($P = 0.048$) in the periodontitis group were significantly higher than the group without periodontitis, but the mean BOP and PI did not differ significantly between the two groups. Pearson correlation coefficient showed a direct and meaningful correlation between PD and anxiety score ($r = 0.369$ $P < 0.001$), and also, PD ($r = 0.363$, $P < 0.001$) and anxiety score ($r = 0.325$, $P = 0.002$) had direct and meaningful correlations with SCL. To determine whether the relationship between PD and SCL was due to anxiety or not, by controlling the confounding effect of anxiety (partial correlation test), we concluded that there was a direct and meaningful relationship between PD and cortisol ($r = 0.277$, $P = 0.01$). Table 2 shows the mean SCL, PD, BOP, and PI in both the groups according to the severity of anxiety.

In the group with periodontitis, 27 (60%) patients had mild periodontitis, 14 (31.1%) had moderate periodontitis, and 4 (8.9%) had severe periodontitis.

We found a strong correlation between PD and severity of the disease ($r = 0.454$, $P < 0.001$, Spearman correlation coefficient). No significant difference between the severity of CP subgroups (mild, moderate, and severe) and SCL ($P = 0.895$) was found [Table 3].

Using the receiver operating characteristic curve, a cutoff point of cortisol of 4.1 ng/ml was considered in the 1.1–10.12 ng/ml range, and accordingly, the members of each group were divided into two categories: SCL more than average (>1.4) and lower than average (≤1/4) [Figure 2]. As Table 4 shows, 62.2% of patients with periodontitis had cortisol levels >4.1 ng/ml, while in patients without periodontitis, this percentage was 44.4%, and people with cortisol level of >4.1 ng/ml were twice more prone to CP disease than those with <4.1 ng/ml cortisol level (odds ratio = 2.06).

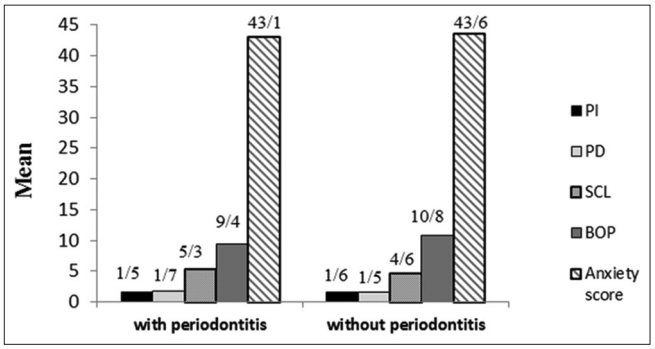

Figure 1: Comparison of salivary cortisol level, probing depth, bleeding on probing, and plaque index and anxiety scores in both the case and control groups.

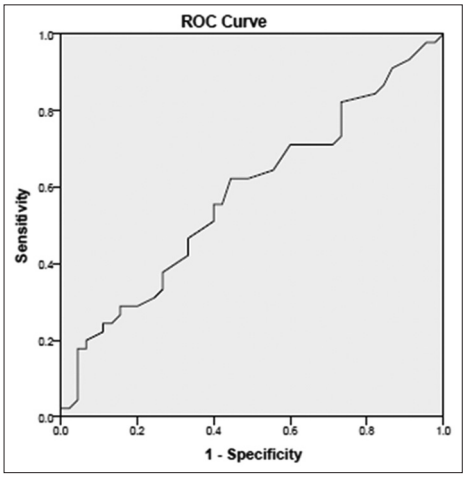

Figure 2: Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.

Table 1: Distribution of the participants according to sex, level of education, and frequency of daily brushing

Groups	Sex		Level of education				Frequency of daily brushing			
	Male (%)	Female	< diploma	Diploma	Associate and bachelor	Master's degree or higher	0 time	1 time	2 time	3 time
With periodontitis	9 (20)	36 (80)	9 (20.9)	19 (44.2)	11 (25.6)	4 (9.3)	4 (8.9)	20 (44.4)	17 (37.8)	4 (8.9)
Without periodontitis	5 (11.1)	40 (88.9)	1 (2.3)	6 (13.6)	18 (40.9)	19 (43.2)	0	28 (62.2)	15 (33.3)	2 (4.4)
P	0.245				0.407				0.672	

Table 2: Mean and standard deviation of salivary cortisol level, probing depth, bleeding on probing, and plaque index in patients with and without periodontitis according to the severity of anxiety

Group	Anxiety level	Mean±SD			
		SCL	PD (mm)	BOP	PI
With periodontitis	Low	4.73 (2.66)	1.5 (0.24)	8.6 (5)	1.4 (0.305)
	Moderate	4.45 (1.91)	1.5 (0.32)	8.2 (7.1)	1.5 (0.378)
	Severe	6.80 (1.91)	2 (0.55)	11.4 (5)	1.6 (0.243)
Without periodontitis	Low	3.89 (2)	1.4 (0.27)	9.3 (6.7)	1.6 (0.558)
	Moderate	3.75 (1.38)	1.4 (0.24)	13.7 (9)	1.5 (0.279)
	Severe	6.31 (1.98)	1.6 (0.25)	9.3 (6.3)	1.5 (0.382)

SD: Standard deviation; SCL: Salivary cortisol level; PD: Probing depth; BOP: Bleeding on probing; PI: Plaque index

Table 3: Salivary cortisol levels according to the severity of chronic periodontitis

CP	Mean of cortisol level	SD of cortisol level	*P*
Mild	5.18	2.05	0.895
Moderate	5.5	3	
Severe	5.4	2.7	

CP: Chronic periodontitis; SD: Standard deviation

Table 4: Classification of each group based on the salivary cortisol level

SCL	CP, *n* (%)	
	With periodontitis	Without periodontitis
> average >4.1	28 (62.2)	20 (44.4)
< average ≤4.1	17 (37.8)	25 (55.6)
Total	45 (100)	45 (100)

CI=1.14-3.73; OR=2.06; *P*=0.012. OR: Odds ratio; CI: Confidence interval; SCL: Salivary cortisol level; CP: Chronic periodontitis

DISCUSSION

The results of this study showed that SCL was different between the two groups with and without periodontitis. Periodontitis is one of the most important and most prevalent diseases of the oral cavity. It is a multifactorial disease, and periopathogenic bacteria are the main cause for the onset of inflammation, but the progression and severity of the disease may be affected by conditions that affect the host immune responses.[28-30] Stress has a direct effect on the immune system, and cortisol is a stress-related hormone.[8]

The results indicated that SCL in patients with periodontitis was significantly higher than patients without periodontitis. Furthermore, the mean PD in the case group was significantly higher than the control group, but the average BOP and PI were not significantly different between the two groups.

Genco *et al.*[10] were the first researchers who investigated two groups of patients (with and without periodontitis) and observed that basal SCL was elevated in the group having periodontitis; however, few details were defined about this relationship in that research. Our findings are similar to Rosania *et al.*'[31] study; they found a direct and meaningful and colleagues[19] did not find a significant correlation between serum cortisol level and periodontal disease. Possible reasons could be the small sample size and age of <30 years. In this study, limitations have been eliminated as much as possible by measuring SCL, studying more samples, and extending the age range, which may encompass patients with more anxiety. This inconsistency and lack of correlation between SCL and periodontal disease were also observed in another study,[20] which might be due to limiting the study to only women, lack of similarity, and disproportionate number of samples in the two groups of case (43 women with stress-related depression) and control (29 controls), while in the present study, in order to unify the severity of anxiety, we have selected an equal number of people in both main groups and each subgroup of anxiety. Furthermore, according to the results, the mean age, sex, level of education, and frequency of daily brushing were not significantly different between the two groups, indicating a similarity between the two study groups.

Some mechanisms have been proposed to describe the relationship between stress and periodontal disease, including (1) psychoneuroimmunologic and immunosuppressive effects and (2) behavioral changes.[31] Behavioral changes such as poor oral hygiene, poor dietary habits, smoking, and alcohol consumption can be harmful for the periodontium.[31] In this study, related demographic questions were also asked for these items, and due to the lack of significant differences between the two groups, it can be concluded that in this study, the role of stress on periodontitis is somewhat more related to the psychoneuroimmunologic mechanism and defines the relationship between cortisol and periodontitis in another way. Secretion of stress hormone, cortisol, increases blood glucose level, activates anti-inflammatory and anti-stress mechanisms, and inhibits the immune responses and production of cytokines.[17] Another function of this hormone is to inhibit T-cell immune responses, which changes the system into humoral immunity and causes the

growth of microorganisms that are stopped by cellular immune responses.[32]

Local tissue is destructed due to the consistent activation of cellular immune response during active periodontal disease.[33] Inflammatory periodontal diseases are associated with increased local and systemic pro-inflammatory cytokines (tumor necrosis factor-alpha and IL-6) and prostaglandins. Such factors play a role in tissue destruction by collaboration of matrix metalloproteinases.[34,35] Stress causes the imbalance of pro-inflammatory and anti-inflammatory responses and might be associated with periodontal disease through change in GCF IL-1 and IL-6 levels, reduction in polymorphonuclear leukocyte chemotaxis and phagocytosis, and reduced proliferation of lymphocytes.[36]

In this study, PD as one of the periodontal indicators had a direct and meaningful correlation with SCL. Some studies also linked SCL with the number of missing teeth and probing PD and stated that high levels of cortisol in circulation (hypercortisolemia) were associated with severity of periodontal disease. Furthermore, in this study, scores of STAI questionnaire had a direct and meaningful correlation with SCLs. The amount of anxiety measured by this questionnaire refers to individual differences in response to various stressful situations. In one study,[37] cortisol level had a significant relationship with severity of periodontitis, although it was not associated with mental stress and results of Lipp questionnaire. Other researchers[38] did not find a significant correlation between salivary cortisol and points of HADS test (Hospital Anxiety and Depression Scale). It seems that this lack of communication is caused by an intervening effect of the coping behaviors (individual methods of dealing with stress) on individual stress and their responses to tests.[39] The coping process acts as an intervening strategy to adjust and reduce sustained stress. As a result, HPA axis secretes less cortisol due to employed strategy (copings).[40] The relationship between severity of periodontitis and SCL has not been studied in detail, but different studies have shown a significant correlation between severity of periodontitis and SCL. Nevertheless, in this study, there were no significant differences between the severity of CP in subgroups according to the CAL (mild, moderate, severe periodontitis) and SCL. This may be due to the low number of patients with severe (n = 4) and moderate (n = 14) periodontitis compared with those with mild periodontitis (n = 17). Further studies are needed to explore this relationship.

Our investigation was an analytic cross-sectional one, so the assessment of psychological state and lifestyle through questionnaires is not completely accurate that is one of the limitations of this research. Environmental conditions and circadian rhythm affect salivary cortisol which in this study was kept under control as much as possible by sampling during a specific time in the morning. To obtain more conclusive results, cohort or empirical studies with long-term follow-up of disease progression and severity need to be designed. Furthermore, evaluation of more samples and selection of individuals under high-stress job conditions is recommended.

CONCLUSION

The results of this study show that there is a correlation between increased SCL and periodontal disease; it seems that people with high levels of cortisol have an increased risk of periodontitis. However, further research in this area is needed.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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