Research Article

Evaluation of Association between Perceived Stress, Anxiety, Depression, Salivary Alpha-Amylase and Salivary Cortisol with Chronic Periodontitis

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Abstract

Introduction: Chronic Periodontitis is a multifactorial inflammatory disease, which is caused by certain series of bacteria in oral biofilm and leads to teeth supporting tissue destruction. Stress, depression and anxiety can affect periodontium health through variable different mechanisms. Cortisol is secreted from adrenocorticotropic glands in pituitary gland and can be used as index to determine psychological stress rate. Alpha-amylase is an salivary gland enzyme which is regulated through sympatric nervous system. The aim of the following study is to compare between different biomarkers related to the emotional stress, anxiety and depression in saliva and their role on periodontal health and also the correlation between periodontal parameters, stress, depression and anxiety.

Method: 90 patients with age ranged from 35 to 45 years were participated in the study. Salivary cortisol and alpha-amylase levels were measured in them. Levels of Stress, Anxiety and Depresion were evaluated using questionnaires. The relationship between psychological and biochemical factors with chronic Periodontitis were assessed. The results were analyzed by statistical analysis.

Results: The mean concentration of salivary cortisol was 7.87±4.37 ng/ml in the case group and 4.33±2.11 ng/ml in the control group. The difference was statistically significant (p<0.001). The mean concentration of salivary alpha-amylase was 3569.27±101.22 U/L in the case group and 3343.44±171.09 U/L in the control group. The difference was statistically significant (p<0.001). The mean stress score was 24.28±3.09 in the case group and 22.96±3.61 in the control group. The difference between two groups was statistically significant (p<0.001). The difference between two groups was statistically significant (p<0.001). The mean anxiety score was 44.11±4.59 in the case group and 36.47±4.30 in the control group, which was statistically significant (p<0.001). The mean depression score was 19.96±3.58 in the case group and 13.13±3.91 in the control group. The difference between two groups was statistically significant (p<0.001).

Conclusion: The findings of these study showed that psychological factors like emotional stress, depression and anxiety and biochemical factors including salivary cortisol and alpha-amylase are higher in patients with moderate to severe chronic periodontitis in comparison to healthy people. also Anxiety and depression are stronger predictors of periodontitis than acute stress and cortisol.

Keywords: Chronic periodontitis; Perceived stress; Anxiety; Depresion; Alpha-amylase; Cortisol

Introduction

Chronic Periodontitis is a multifactorial inflammatory disease, which is caused by certain series of bacteria in oral biofilm and leads to teeth supporting tissue destruction. Progress and intensity of disease is related to the host response and environmental factors. Systemic condition, certain habits, smoking and psychological stress are mentioned as the etiologic triggering factors in disease pathogenesis [1]. Epidemiologic studies suggested that between 5 to 20% of world population has the signs and symptoms of severe periodontitis. Furthermore, 35% of teeth extractions reports were because of periodontal diseases [2]. Stress, depression and anxiety can affect the health of periodontium through variable mechanisms. Tissue damage resulting from psychological stress can be variable based on difference in host response toward emotional stress. Ineffective response to stress can play a vital role in progression of periodontal disease and so preventive efforts should be taken in these cases [3,4]. Evaluation of stress, anxiety and depression can be performed *via* questionnaire, which determines the rate of these parameters and also individuals' response and subsequently their implications on oral health. Evaluation of oral fluids biomarkers is a objective and alternative tool to evaluate stress. Oral saliva with more than 3000 proteins and

10000 bacterial species is a good diagnostic resource [5,6]. Saliva examination is a novel non-invasive diagnostic tool, which can be used to detect various diseases specially oral diseases [7-9]. Although Stress, Anxiety and depression are risk factors for periodontitis but their exact pathogenesis are unknown. Stress can suppress immune response   three different paths: Hypothalamus-Pituitary-Adrenal path, peripheral diffusion of neuropeptides and autonomy nervous system [10,11]. A certain biomarker in HPA path is cortisol, which secreted from adrenocorticotropic glands in pituitary glands and can be used as index to determine psychological stress rate. The salivary cortisol level is equal to its level in serum and so can be reflected for HPA path efficacy [12]. Some studies have emphasis on the correlation between salivary cortisol level, periodontitis and alveolar bone loss and proved the relation between cortisol and periodontitis [13,14]. Alpha-amylase is an salivary gland enzyme which is secreted through sympatric nervous system. This salivary protein is synthesized in acinar epithelial cells [15]. Alpha-amylase can reflect psychological stress and is a special marker to determine central nervous system activity. Autonom nervous system action can affect salivary glands and control alpha-amylase secretion [16]. Several other studies have emphasized on Alpha-amylase role under psychological stress [17]. Previous studies suggested the relation between salivary markers to emotional stress and Periodontitis [18,19]. The aim of the following study is to compare between different biomarkers related to the stress, anxiety and depression in saliva and their role on periodontal health and also the correlation between periodontal parameters, stress, depression and anxiety. Change in these biomarkers and the amount of stress, anxiety and depression in patients with periodontitis, supports the hypothesis that emotional stress, anxiety and depression are risk factors for periodontal disease.

Methods

The study protocol was admitted and confirmed by the Medical ethics committee of Babol university of medical science, Babol, Iran (Code: 087. 2019). In this case-control study, 90 patients (45 with CP and 45 healthy participants as control group) with different ages ranging from 35 to 45 years referred to periodontology ward of Babol dentistry faculty, Babol, Iran. The participants in both groups were standardized both in gender and age range.

Patients with generalized moderate to severe chronic periodontitis according to criteria of American Periodontology Academy (APA) were included and examined by a calibrated periodontist. Chronic periodontitis is defined as moderate to severe when we have bleeding on probing and clinical attachment loss more than 2 mm. Healthy individuals were chosen from Oral medicine ward of Babol dentistry faculty. They had no Probing Depth (PD) ≥3 mm and had no bone loss [20]. Exclusion criteria were as follows: history of periodontal treatment in recent 6 months, systemic disease, antimicrobial treatment in last three months, smoking and alcoholism, inability to write and respond to questionnaire, pregnant and breastfeeding women, immunosuppress patients, steroid consuming, Neuropsychotherapy, inability to respond to the questionnaire because of psychological disorders. After taking written consent form, questionnaires related to stress, anxiety and depression evaluation were responded by the participants. Non-irritated saliva sampling were conducted by using Spitting technique in 9:00 to 11:00 AM. Patents were sited, bowing his head forward.

immediately before sampling the patient was requested to gulp his remained saliva and keeping his spittle on his mouth for some seconds and then gently spitted 1 to 1.5 mL of that on a examination tube which was located just below his chain. All samples including freezing medium transferred to the biochemistry ward of Babol university of medical science, and centrifuged in a speed of 1500 rpm for 15 min in order to separate the cellular debris and then the specimen were stored in -20ºC temperature. Evaluation of stress level was conducted using Cohen questionnaire. This questionnaire was first published in 1983 by Cohen et al. this questionnaire is composed of 14 questions, which measures the level of stress of the individual in last month. In this questionnaire a five-type laicert questioned were used, the answers were variable from never, rare, sometimes, often, always. The scores were ranged from 0 to 4. Reliability of the questionnaire was confirmed using Intracorrelation coefficient and alpha-Cruhnbach coefficient of 0.84 to 0.86. Ghorbani et al., in another study evaluated the validty and reliability of this questionnaire and reported its Alpha-cruhnbach coefficient 0.81 for Iranian and 0.86 for American respondents [21]. Emotional depression was evaluated using Back questionnaire (2nd ed). Back et al., first published this questionnaire. This questionnaire has a 21 items, each item has a 0 to 3 score range, the total score level is between 0 to 63. Each item evaluates one of the depression parameters. Validity and reliability of this questionnaire was confirmed by a study conducted by Ghasemzadeh et al., by Alpha-cruhnbach coefficient of 0.087 and test re-test coefficient of 0.74 and its correlation with first edition of questionnaire was 0.97 [22]. For anxiety, we used Spill-burger questionnaire, which has 40 items, this questionnaire has 40 items consisting of two different parts: state anxiety and trait anxiety, which each one was analyzed using 20 items. In this study, we used trait anxiety. Each item has four choices: 1) rarely 2) sometimes 3) often 4) always. Validity of this questionnaire was reported as 0.73 to 0.86. Alpha coefficient for this questionnaire for different groups was reported to be 90% [23].

To evaluate salivary alpha-amylase level, 1 mL of substrate was placed for 5 min in 37ºC. Then 20 μL of each centrifuged sample were added. After mixing, the samples were stored in Benn-Mary in a 37ºC temperature for 7.5 min. Then, chemical reaction ceased using 1 mL iodine solution and 8 mL distilled water. Salivary samples were added to all tubes except blank tube. Absorbance rate was interpreted in 660 nm wavelength against distilled water.

Salivary Cortisol level was measured using Human Saliva Kit (ZellBio GmbH Germany, Cat No: ZB-S11003-H9648) with a 1 ng/mL sensitivity. In this study, ELISA were used on Competitive technique so as at first 50 μL of control, standard and salivary samples were purred in the related wells. Then 100 mL of conjugate were added to the wells for marking atigen-antibody reaction and incubated in 37ºC for 45 min in thermoshaker. Then each wells was washed 3 times with 300 μL of wash Buffer solution to discharge non-special antigen and antibodies. Then 150 mL of TMD substrate were added to the wells to react with conjugate and the products were monitored as color in wells. Then the specimen were incubated in a thermoshaker for 20 min and then 50 μL of stop solution was added to the wells to stop enzymatic reactions. Eventuallty absorbance were read using ELISA reader (RAyto Co, Xan, China) in 450 nm wavelength. All data were recorded in SPSS (IL Chicago, USA) version 24.0 Statistical Analysis conducted using t-test, Chi-square test, linear regression and logistic

regression. Significance relation was mentioned as p-value lower than 0.05.

Results

90 patients with age ranged from 35 to 45 years were participated in the study. From them 45 patients had moderate to severe Chronic periodontitis and 45 were healthy people. The participants all responded to the questionnaires and Salivary cortisol and alpha-amylase were measured using ELISA and spectrophotometry method respectively. Each group was standardized and the men to women ratio was equal in both groups (23 men and 22 women) (Table 1). Level of Salivary cortisol were measured in both groups, the results showed that the mean concentration of salivary cortisol was 7.87±4.37 ng/ml in the case group and 4.33±2.11 ng/ml in the control group. The difference between two groups was statistically significant (p<0.001). Level of Salivary Alpha-amylase was measured in both groups, the results showed that the mean concentration of salivary alpha-amylase was 3569.27±101.22 U/L in the case group and 3343.44±171.09 U/L in the control group. The difference between two groups was statistically significant (p<0.001). Score of Stress was measured and recorded with questionnaire, the results showed that the mean stress score was 24.28±3.09 in the case group and 22.96±3.61 in the control group. The difference between two groups was statistically significant (p<0.001). Score of anxiety was evaluated using questionnaire. The results showed that the mean anxiety score was 44.11±4.59 in the case group and 36.47±4.30 in the control group, which was statistically significant (p<0.001). Score of depression was evaluated using questionnaire. The results showed that the mean depression score was 19.96±3.58 in the case group and 13.13±3.91 in the control group. The difference between two groups was statistically significant (p<0.001). Linear regression model was used to evaluate the role of sex and education status. The results suggested that alpha-amylase and cortisol level in men was more than women, but the difference was not statistically significant (p=0.15 for alpha-amylase and p=0.35 for cortisol). Also there was no significant difference in alpha-amylase level between different educational status (p>0.05). For cortisol there was significant difference in different educational status (p=0.04). Patients graduated from diploma degree had lower salivary cortisol level than under graduated participants (p=0.009). Also there was no significant difference in stress, depression and anxiety between two genders (p>0.05). Also there was no statistically difference in anxiety and stress with educational status (p>0.05). Patients graduated with B.C degree and upper had a lower depression from others (p=0.009). Logistic regression model was used to evaluate which study variables are the strongest predictors of periodontitis and it was observed that acute stress is not a strong predictor of chronic periodontitis (OR=1/23). But when stress becomes chronic and turns into anxiety and depression, it becomes a stronger predictor of periodontitis. Anxiety is a stronger predictor of periodontitis than acute stress (OR=1/40). Depression is also the strongest predictor of periodontitis among the variables studied in this study (OR=2/51). Cortisol (OR=0/36) is a weaker predictor of periodontitis than alpha-amylase (OR=1/01).

Discussion

This study was conducted in order to evaluation the correlation between biochemical factors including salivary cortisol and alpha-

Table 1: Sex and education status distribution between participants.

Group		Case	Control	p-value*
Sex	Male	23 (51)	23 (51)	1
	Female	22 (49)	22 (49)	
Education Status	Under graduated Diploma	11 (24.4)	14 (31.1)	0.73
	Graduated Diploma	25 (55.6)	24 (53.3)	
	B, C and upper	9 (20.0)	7 (15.6)	

*Chi Square test.

amylase and psychological factors including psychological stress, anxiety and depression with chronic periodontitis. As the increasing prevalence of periodontal disease and their role on tooth loss, Prevention, diagnosis and treatment of these disorders has great importance. According to the findings of the following study, biochemical factors including salivary levels of cortisol and alpha-amylase in patients with moderate to severe chronic periodontitis was significantly higher than healthy people. Also the level of psychological factors including stress, anxiety and depression was significantly higher in patients with moderate to severe chronic periodontitis. Although the T-test showed that stress and cortisol are higher in people with periodontitis, logistic regression showed that stress becomes a stronger predictor of periodontitis when it becomes chronic and manifests as depression and anxiety. The difference between acute and chronic stress is important to consider. In this way, acute pressures lead to a short-term response, and in this case, an effective solution is found for the needs and threats. In contrast, chronic stress is long-term or due to a lack of effective solutions to needs and threats. Adverse health outcomes occur when threats and needs are significantly imposed or exceed a person's capacity to respond to these factors [24]. Stressful situations trigger a range of emotional reactions, from excitement to anxiety, anger, frustration, and depression. If the stressful situation continues, the emotions will change between these reactions according to the individual's coping efforts [25]. In higher educational status, the level of salivary cortisol was reduced significantly but there was no difference in alpha-amylase level between different educational status. Cortisol is secreted through adrenal glands. This hormone secreted during stress and psychological imbalance and leads to immunosuppression. So the individual is susceptible to different physical-mental and inflammatory disease which makes individual, a good candidate for periodontal disease [26]. Alpha-amylase is an enzyme which is secreted from acinar cells located in epithelial base of salivary glands in response to sympathic nervous system induction [14]. This enzyme is secreted in response to stress and psychological factors and is a good biomarker to determine central nervous system activity [15]. According to our findings we can conclude that this parameter can be chosen as an index to determine the intensity of psychological stress and to reduce their burden and eventually periodontal diseases. Any Rosania E et al., in a study conducted in 2009, concluded that there was a correlation between depression, salivary cortisol and periodontal disease [18], which was in agreement with our findings. Balwan Rai et al., in another study concluded that there was a correlation between biochemical and clinical biomarkers [2], which was in accordance with our findings. The positive point of the following study in comparison to the similar studies is the simultaneous correlation between psychological factors with present biochemical factors.

Jaswail R et al., in another study in 2016 concluded that there was a correlation between serum cortisol level, emotional stress and chronic periodontitis [27], which was in accordance with our findings. Haririan et al., in another study in 2017 evaluated the correlation between multiple factors in saliva and serum with stress and periodontitis. The results showed that there was no correlation between chronic and aggressive periodontitis with salivary and serum level of cortisol [3]. This findings was in contrast with our findings. The reason for this inconsistency was related to difference in methodological design of the relevant studies.

Conclusion

The findings of these study showed that psychological factors like emotional stress, depression and anxiety and biochemical factors including salivary cortisol and alpha-amylase are higher in patients with moderate to severe chronic periodontitis in comparison to healthy people. Also Anxiety and depression are stronger predictors of periodontitis than acute stress and cortisol.

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