Original Article

Effect of Psychological Stress on Salivary Cortisol and Periodontitis

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Abstract

Objective: The progression of periodontitis, induced by polymicrobial dysbiosis, can be modified by systemic or environmental factors such as stress or anxiety that affect host response. This study evaluates the potential associations between psychosocial stress, salivary cortisol and periodontitis.

Methods: In this cross-sectional study, 80 adult participants (41 males and 39 females) aged 20-45 years were included. Participants completed a stress self-assessment using a PSS scale questionnaire. Samples of saliva were collected for testing cortisol levels by ELISA. The participants were then divided into four groups established on periodontal parameters (plaque index (PI), bleeding on probing (BOP), probing pocket depth, and clinical attachment level), and stress levels: Group 1 (healthy periodontium without stress), Group 2 (periodontitis without stress), Group 3 (healthy periodontium with stress), and Group 4 (periodontitis with stress).

Results: Statistically significant differences were observed between the values detected in the four experimental groups for PI, BI, PD, and CAL ($p \leq 0.05$). The highest means of cortisol level were revealed in the stressed healthy group and stressed with periodontitis group, at 39.7 and 40.5, respectively. Hence there were statistically significant differences overall across the four groups ($p \leq 0.05$).

Conclusions: This study demonstrates that psychosocial stress is a risk factor for periodontal diseases, and in cortisol, as one of the elements that enhance periodontal damage, increases were recorded for all four clinical parameters, BI, PI, PD, CAL, which are used as diagnostic tools for periodontitis.

Keywords: *Psychological stress, Periodontal disease, and Salivary cortisol levels.*

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Introduction

Periodontal diseases are long-term inflammatory conditions affecting the tissues surrounding, supporting, and protecting teeth[1]. Periodontitis can be described as multifactorial chronic inflammation, which is characterized by clinical signs that may include visible or unsightly inflammation, spontaneous or induced gingival bleeding of varying severity, the development of pockets related to bone attachment losses, alveolar bone losses, tooth movement, and may result in the loss of teeth[2]. Periodontitis is admitted and generated by a complicated interaction linking bacterial biofilm and the host defense system[3]. There are many local and systemic risk factors, including sex, genetics, age, race, socioeconomic status, poor oral hygiene, smoking, improper diet, hormones, taking particular drugs, and many diseases, such as AIDS, diabetes, and osteoporosis[4]. Those factors influence the inflammatory response's evolution and cause the elevation of proinflammatory cytokine that induces tissue destruction and tooth loss[1]. Accordingly, psychosocial stress has become recognized as a risk factor, with numerous types of research showing a connection between psychosocial stress and different types of periodontal disease[5]. Psychological stress affects immune response shifts, improving periodontitis susceptibility[6].

Stress is a state of psychological or physiological force affected by opposing provocations, such as mental, emotional, physiological, external, or internal, that work to interrupt an organism's functioning and which the organism desires to avoid[7]. Stress or anxiety is widely known as an objective factor that can instantly negotiate periodontal disease through numerous biological or behavioral mechanisms when they become extreme and chronic[8]. Stress is correlated with the predisposition, precipitation, perpetuation, and aggravation of various diseases and worsening morbidity and mortality[9]. Inflammation has been proven to be triggered by stress and exacerbated in situations with infectious, allergic, autoimmune, or neoplastic etiologies, as well as cardiovascular, metabolic, digestive, pulmonary, and rheumatologic diseases[10].

To clarify the mechanisms through which stressors render a physiological response from the immune, endocrine, and nervous systems, a theoretical study by G. Slavich stated that "Two physiological pathways are capable of transforming social, environmental adversity into large proinflammatory transcriptional programs. The sympathetic nervous system is a part of the first pathway (SNS), and the second pathway comprises the hypothalamic-pituitary-adrenal (HPA) axis"[11].

Cortisol, a glucocorticoid stress biomarker, is responsible for maintaining the organism's homeostasis[12]. Nevertheless, an aggravated production of cortisol can result in nocive outcomes, such as deregulation of the immune reaction and shifts in inflammatory modulation. The hypothalamic–pituitary–adrenal axis produces cortisol from stress, which initiates this response in the central nervous system[13, 14].

In the long term, cortisol can reduce the immune system's ability by hindering immunoglobulins A and G, changing the T-helper and T suppressor balance and inducing modifications of natural killer cells[15, 16]. It has been determined that psychological stress may play a role in the etiology of diseases like periodontitis due to the combination of inflammatory reactions and immune system suppression brought on by increased cortisol levels in the body[17]. The present study attempted to evaluate the interrelationships between salivary cortisol concentrations, self-reported stress and periodontal clinical parameters.

Patients and methods

Patients

This clinical study was carried out in the Department of Periodontics at the College of Dentistry and Periodontics Clinic at Shorsh Dental Center and Piramerd Dental Center, with assistance from the Department of Psychiatry at Ali Kamal Hospital and a private consultant clinic in Sulaimani City, north of Iraq. After getting permission from the Ethical Committee of the College of Dentistry at the University of Sulaimani, the essence of the study was clarified to all the participants, and written informed permission was obtained. A total of 300 participants were screened for six months. According to the current periodontal conditions and illnesses classification, moderate to severe periodontitis corresponds to stages–IV/grades A–C[18]. Participants without systemic diseases (e.g. diabetes), not taking an anti-inflammatory, antimicrobial or other drugs affecting immune status or the bacterial ecosystem (such as channel blockers and anti-epileptics), plus non-pregnant, non-smokers, non-alcohol users, and those who had not undergone periodontal therapy in the six previous months[19], were considered as candidates for the study. Using the above inclusion and exclusion criteria for both sexes, only 80 people, ranging from 20 to 45 years, were included in the final study. The included participants were separated into four groups based on their periodontal examinations and stress assessments: Group 1 (healthy periodontium without stress, 20 participants), Group 2 (periodontitis without stress, 20 participants), Group 3 (healthy

periodontium with stress, 20 participants), and Group 4 (stress and periodontitis, 20 participants).

Clinical evaluation and salivary sampling

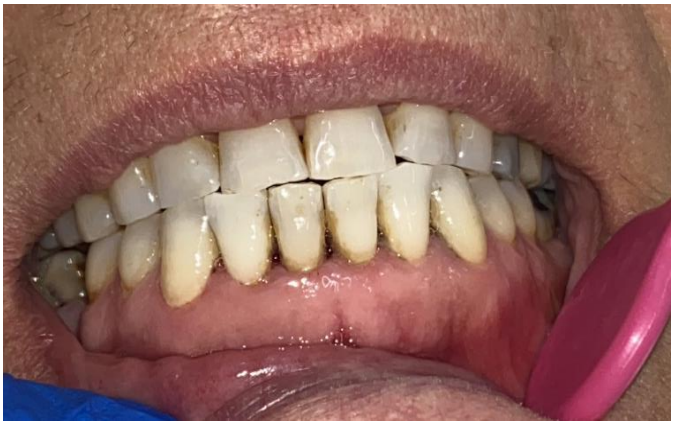
The same calibrated operator examined all participants. Unstimulated saliva was collected to estimate salivary cortisol levels; collected samples were immediately centrifuged and frozen at −80 ◦C until analysis[20], when the following four periodontal clinical parameters were meticulously recorded for all the participants' teeth: (i) periodontal pocket depth (PPD) or probing depth, (ii) level of clinical attachment (CAL), (iii) presence of bleeding on probing (BoP), and (iv) plaque index (PI)[8]. As shown in Figure 1.

Psychosocial Stress Measurements

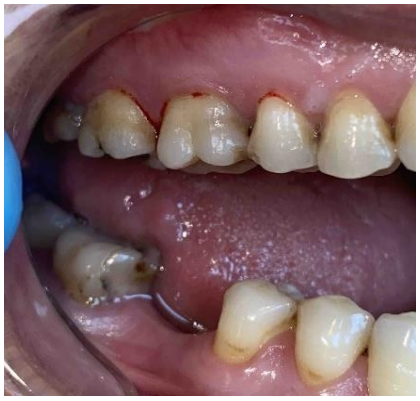
The PSS scale was used, which measures each patient's perception of stress in the last month and whether they were able to manage it. It is a questionnaire comprising 10 questions, each rated on a scale ranging from 1 to 5 points. High scores suggest high stress levels: unstressed (<21), managed stress and very stressed (>27)[21].

Statistical analysis

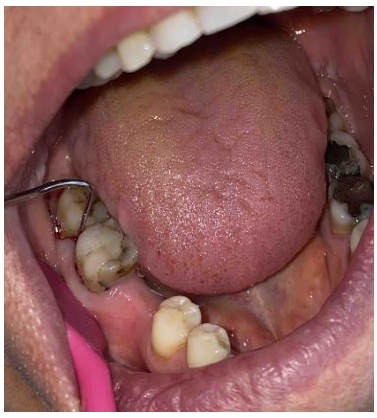
To characterize the findings of the study for each parameter examined, descriptive statistical analysis was employed. Continuous distribution variables' means, and standard deviations were displayed, as well as the counts and percentages of categorical variables. The Mann-Whitney U test was utilized to define whether the differences between the two independent groups and within-group differences were statistically significant. The Kruskal-Wallis test was used to determine this for the four experimental groups. The chi-square test was used to evaluate theories concerning associations between categorical variables. Pearson correlation was used to calculate the correlation between the different variables. A p-value of 0.05 or less was employed to assess the statistical significance. In all tests, statistical significance was defined as a p-value of 0.05 or less. SPSS for Windows 27.0 was applied for statistical analysis.



A: Periodontal examination for plaque frontal view



B: Examination for bleeding buccal view



C: Examination for pocket depth buccal view pd = 5mm

D: Examination of CAL for lower anterior lingual view and upper six palatal view

Figure 1: Periodontal examination for periodontitis patient.

Results

Table 1 shows demographic data for the different groups, the mean and standard deviations of age. The average ages of the non-stressed healthy group, non-stressed with periodontitis group, stressed healthy group, and stressed with periodontitis group were (34.5, 34.5, 32.4 and 36.9) respectively. The distributions of gender in the different groups were approximately equal. The p-value for further testing for variables between groups was of greater significance than 0.05, suggesting no difference in selecting samples. To put it another way, this indicates that there was no bias in sample selection, and proper randomization was used. The table is put in the appendix as table one.

Table 2 summarizes the distribution of the clinical characteristics analyzed in participants' saliva. PI revealed that the highest mean levels were in the periodontitis group with stress (0.26±0.05), BI, PD, and CAL revealed that the highest mean levels were in G4 (0.17±0.03,4.99±0.53, 3.72±0.49) respectively.

Statistically significant differences were observed between the values detected in the four experimental groups for PI, BI, PD, and CAL (P≤0.05).

Cortisol testing revealed that the highest mean levels were in the stressed healthy group and stressed with periodontitis group, at 39.7 and 40.5, respectively, which means that there were statistically significant differences in the mentioned measurements across the four groups.

Table 3 displays the mean differences between each pair group for all variables listed. In PI, BI, PD, and CAL all differences between pair groups are statistically significant (P≤0.05), except for the mean differences between the non-stressed healthy and the stressed healthy group (P≥0.05). This is because there is no relevant data for CAL in the non-stressed healthy and stressed healthy groups. Cortisol testing showed all differences between pair groups to be statistically significant (P≤0.05), except for the mean differences between the non-stressed healthy group and non-stressed with periodontitis group and the stressed healthy group and stressed with periodontitis group, which are not statistically significant (P≥0.05).

Table 4 summarizes the variable-to-variable correlations for the non-stressed healthy group. PI was positively correlated (intermediate) with BI (r = 0.603) and cortisol (r = 0.545), BI-cortisol (r=0.516). In the non-stressed with periodontitis group, PI was positively correlated (intermediate) with BI (r = 0.683) and PD (r = 0.447) in the stressed healthy group. BI was negatively correlated (intermediate) with cortisol (r = -0.539) in the stressed with periodontitis group, and PI was positively correlated (intermediate) with PD (r = 0.536).

Table 1: Demographic data for the different groups.

Group(n)	Age (year)	p value[a]	Gender		p value[b]
	mean(SD)		Male,n(%)	Female,n(%)	
G1(20)	34.5(8.25)	0.388	11(55)	9(45)	0.908
G2(20)	34.5(8.19)		11(55)	9(45)	
G3(20)	32.4(8.93)		9(45)	11(55)	
G4(20)	36.9(7.22)		10(50)	10(50)	
Total(80)	34.575(8.17)		41(51.2)	39(48.8)	

Table 2: Clinical characteristics of the experimental groups (mean ± SD)

Variables	Group(n)	Mean	SD	Mean Rank	Kruskal Wallis Test
PI	G1(20)	0.1445	0.02523	28.28	H=41.62 Df=3 p value=0.000
	G2(20)	0.2265	0.07782	50.25	
	G3(20)	0.1563	0.02549	20.88	
	G4(20)	0.267	0.05192	62.6	
	Total(80)	0.1986	0.07064		
BI	G1(20)	0.0565	0.02254	20.42	H=50.322 Df=3 p value=0.000
	G2(20)	0.1385	0.06862	51.4	
	G3(20)	0.066	0.01273	25.28	
	G4(20)	0.1765	0.03031	64.9	
	Total(80)	0.1094	0.06367		
PD	G1(20)	1.678	0.40896	13.05	H=66.069 Df=3 p value=0.000
	G2(20)	4.4125	0.49143	54.48	
	G3(20)	2.4445	0.53862	27.95	
	G4(20)	4.9985	0.53986	66.52	
	Total(80)	3.3834	1.45813		
Cal	G1(20)	0	0	20.5	H=70.646 Df=3 p value=0.000
	G2(20)	2.8955	0.81166	54.62	
	G3(20)	0	0	20.5	
	G4(20)	3.7245	0.49114	66.38	
	Total(80)	1.655	1.75418		
Cortisol	G1(20)	13.6625	3.17411	17.8	H=59.882 Df=3 p value=0.000
	G2(20)	15.2231	3.09519	23.2	
	G3(20)	39.7519	6.28595	59.62	
	G4(20)	40.5688	4.99086	61.38	
	Total(80)	27.3016	13.71329		

Table 3: Mean differences between independent pair groups.

Variables	Group(I)-Group(J)	Mean Difference (I-J)	p value*
PI	G1-G2	-.08200*	0
	G1-G3	-0.01175	0.13
	G1-G4	-.12250*	0
	G2-G3	.07025*	0.001
	G2-G4	-.04050*	0.028
	G3-G4	-.11075*	0
BI	G1-G2	-.08200*	0
	G1-G3	-0.0095	0.216
	G1-G4	-.12000*	0
	G2-G3	.07250*	0
	G2-G4	-.03800*	0.017
	G3-G4	-.11050*	0
PD	G1-G2	-2.73450*	0
	G1-G3	-.76650*	0
	G1-G4	-3.32050*	0
	G2-G3	1.96800*	0
	G2-G4	-.58600*	0.001
	G3-G4	-2.55400*	0
Cal	G1-G2	-2.89550*	0
	G1-G3	0	1
	G1-G4	-3.72450*	0
	G2-G3	2.89550*	0
	G2-G4	-.82900*	0.001
	G3-G4	-3.72450*	0
Cortisol	G1-G2	-1.56063	0.144
	G1-G3	-26.08938*	0
	G1-G4	-26.90625*	0
	G2-G3	-24.52875*	0
	G2-G4	-25.34563*	0
	G3-G4	-0.81687	0.636

Table 4: Correlation between variables in saliva within variate groups.

Groups	Variables	PI	BI	PD	Cal	Cortisol
G1	PI	1	.603**	0.072	.a	.545*
	BI		1	0.165	.a	.516*
	PD			1	.a	0.224
	Cal				.a	.a
	Cortisol					1
G2	PI	1	.683**	.447*	0.216	0.144
	BI		1	0.288	-0.069	-0.099
	PD			1	0.313	-0.142
	Cal				1	-0.202
	Cortisol					1
G3	PI	1	0.125	0.181	.a	0.017
	BI		1	0.335	.a	0.162
	PD			1	.a	0.242
	Cal				.a	.a
	Cortisol					1
G4	PI	1	-0.051	.536*	0.117	0.408
	BI		1	0.236	0.107	-0.195
	PD			1	-0.18	-0.028
	Cal				1	0.281
	Cortisol					1

*: Mann Whitney U test

a: Cannot be computed because at least one of the variables is constant.

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

Discussion

Several pathologies can affect inflammatory processes and cause inflammatory disorders, and stress and anxiety have been recognized as risk factors for many pathologies[22]. Humans' low-grade inflammation appears to be influenced by acute and chronic psychosocial stress. There may be links between the inflammatory response to acute psychosocial stress and the long-term development of diseases like cardiovascular disease, diabetes mellitus, or periodontal diseases[23].

In this study, PI and BI revealed differences between all groups that were statistically significant, except between groups I and III, where the difference was not statistically significant. Group IV, which represents stage II periodontitis with stress, recorded high mean values of PI and BI compared to other groups, which is identical to the finding by Mannem and Chava[24]. As explained by Goyal *et al* and Rohini *et al.*[25], plaque levels are linked positively with stress and cortisol.

It was noted that the stress factor has a fundamental relation to plaque and periodontal disease, which agrees with our study. These data strongly support the notion discussed by Genco et al.[26] that stress effects on periodontal health might be mediated, at least in part, by stress-induced neglect of oral hygiene for further potential mediators, which might, in part, act synergistically to cause stress-related plaque accumulation.

For PD and CAL, statistically significant differences were observed among all groups, but no statistical difference was found between groups I and III for CAL, with a higher mean value of PD and CAL in group IV, followed by group II, group III, and lastly group I, which is similar to Hilgert *et al*[27]. Good reproducibility was reported for both the PD and CAL measure, caused by psychological stress, which has been linked to periodontitis and has been shown to cause higher IL-1 levels and MMP in people with both illnesses[28]. This

cytokine imbalance alters the host's reaction and resistance to infections, worsening damage in chronic diseases like periodontitis[29].

Comparing salivary cortisol levels between groups revealed statistically significant differences between all groups. Nevertheless, no statistically significant difference was found between groups I and II, III and IV. The higher mean values in group IV were attributed to the superimposition of both periodontitis and psychological stress[30]. This result corresponds positively with the high scores on the perceived stress scale obtained from the study's participants. The next highest was in group III, followed by group II, and lastly group I. This result aligns with Genco et al.[31] who found that the group with periodontitis had higher mean salivary cortisol levels in a sample of individuals with and without periodontitis. More recently, saliva has been used as a potential source for analyzing biomarkers in periodontitis and stress.

Studies have found that cortisol in saliva (1) represents "free" biologically active cortisol, (2) is unaffected by salivary flow rate, (3) consistently and reliably reflects free serum cortisol and HPA axis reactivity, and (4) is also a more practical assessment tool than venipuncture in stress research due to its potential to elicit spurious increases in cortisol secretion reflecting a "hyper stress" component[32].

On the correlation of the periodontal clinical parameters and cortisol, in group I, PI was positively correlated with BI and cortisol, and BI with cortisol; in group II, PI was positively correlated with BI and PD; in group III, BI was negatively correlated with cortisol; in group IV, PI was positively correlated with PD, which indicates that high salivary cortisol is associated with more periodontal destruction[29].

Within the study's limits, we did not measure salivary flow rate, salivary PH, because saliva can be influenced by emotions since stress can create acids in the body. The dry mouth is experienced under extreme stress and with any medications taken to alleviate stress, which decrease saliva production. The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow), while the normal pH of saliva is 6 to 7, meaning that it is slightly acidic[33].

The following are the study's key findings: (i) a positive relationship between cortisol concentrations and results from the stress self-report questionnaires, (ii) a positive linear relationship between cortisol levels in patients with periodontitis and the plaque score, bleeding score, pocket depth, and clinical attachment loss.

Conclusion

Within the study's limits, more periodontal destruction and disease severity were observed in periodontitis associated with stress, showing increased PD, CAL, and disease activity. The levels of this protein were found to be higher in periodontitis associated with stress than in periodontitis or stress alone.

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