# **Original Article**

# Association of stress, salivary cortisol level, and periodontitis among the inmates of a central prison in Kerala

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### ABSTRACT

**Background:** The aim of this cross-sectional study was to evaluate the association between stress, salivary cortisol, and periodontitis among the inmates of the central prison.

**Materials and Methods:** Seventy inmates were grouped depending on their pocket depth into Group A (pocket depth >4 mm and <6 mm), Group B (at least four sites with pocket depth  $\geq$ 6 mm), and Group C (pocket depth  $\leq$ 3 mm). The clinical parameters such as the oral hygiene index-simplified, gingival index, pocket depth, and the clinical attachment levels (CALs) were recorded. Stress was measured using the Depression, Anxiety, and Stress Scale along with prison time served. Saliva samples were collected, and cortisol levels were determined using electrochemiluminescence assay. Chi-square test was used for finding the association between the clinical parameters. The correlation between clinical parameters, stress, salivary cortisol levels, and time served was done using Pearson's rank correlation coefficient.

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Address for correspondence: Dr. Angel Fenol, Department of Periodontics, Amrita School of Dentistry, AIMS, Edapally, Kochi - 682 041, Kerala, India. E-mail: angelfenol@aims. amrita.edu **Results:** The CALs, the stress score and the salivary cortisol levels were significantly higher in Group B (P < 0.001). Pearson's correlation showed a positive correlation between stress, cortisol level, and pocket depth. A positive correlation which was statistically significant was obtained between salivary cortisol level and prison time served by the inmates.

**Conclusion:** Within the limits of this study, it can be concluded that there is a positive relation between stress and periodontal disease. The study suggests that salivary cortisol level can be used as a marker to assess stress.

Key Words: Cortisol, periodontitis, stress

# **INTRODUCTION**

Periodontitis is a multifactorial disease.<sup>[1]</sup> Imbalances in the immune system can be caused by environmental factors such as stress leading to disruption of the equilibrium between tissue destruction and repair.<sup>[2]</sup> Stress is simply a reaction to a stimulus that disturbs the physical or mental equilibrium.<sup>[3]</sup> Problems start when the stress response is inappropriate to

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Website: www.drj.ir www.drjjournal.net www.ncbi.nlm.nih.gov/pmc/journals/1480 the intensity of the challenge. Selve also postulated that the mechanism of action central to stress phenomenology was activation of the adrenocortico pituitary axis.<sup>[4]</sup>

A reasonable amount of research indicates the association of psychosocial stress<sup>[5-7]</sup> financial

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stress, occupational stress, distress, and depression with periodontitis.<sup>[8]</sup> Psychosocial and physical conditions can influence host defences exerting an immunosuppressive effect which increases one's vulnerability to disease.<sup>[9,10]</sup>

The purpose of this cross-sectional study was to investigate a possible relationship between psychosocial stress and periodontal disease. An attempt was made to evaluate the effects of psychological stress on periodontal health by assessing the periodontal status of the inmates of a central prison who were assumed to be under stress. The salivary cortisol level was estimated to ascertain the link between stress and periodontal disease.

### **MATERIALS AND METHODS**

### Study population and sampling

The study was conducted in the hospital wing of Viyyur central jail, and the biochemical analysis was conducted in the biochemical laboratory of Amrita Institute of Medical Sciences. The study was approved by the Ethical Committee of Amrita School of Dentistry and Ministry of Home Affairs, Government of Kerala.

A total of seventy individuals aged between 25 and 60 years, having a minimum number of 20 teeth were included in this study. A written informed consent was obtained from all the participants. Individuals whose health status could interfere with the study, for example, those who were taking corticosteroids or immunosuppressant drugs, those having Addison's disease or Cushing's syndrome, current smokers, individual with systemic conditions that could interfere with periodontal disease, or any history of psychiatric disorder, female individuals and those individuals who underwent periodontal therapy in the past 6 months were excluded from the study.

Individuals were grouped according to probing pocket depth (PPD) into-Group A (PPD  $\geq$ 4 and <6 mm), Group B (PPD  $\geq$ 6 mm) in at least 4 sites and control (PPD  $\leq$ 3 mm).<sup>[11]</sup> The sample size was calculated on the basis of a study by Mahendra *et al.*,<sup>[11]</sup> using 72% prevalence rate of periodontal disease, with 20% allowable error and 95% confidence interval, it was determined as 37. The sample size taken for the study was seventy. Stress evaluation was assessed based on a standardized stress questionnaire and salivary cortisol level.

#### **Evaluation of stress**

The Depression, Anxiety, and Stress Scale (DASS) was used in this study. It is a set of three self-report scales diagnosis<sup>[12]</sup> to measure the emotional states of depression, anxiety, and stress. It has 21 items (seven items for each category) based on a four-point rating scale. Results from various studies have shown that DASS scale shows strong construct validity.<sup>[13]</sup> Each of the three scales contains 7 items, divided into subscales with similar content. Each item is scored from 0 to 3.

- 0. Did not apply to me at all NEVER
- 1. Applied to me to some degree, or some of the times SOMETIMES
- 2. Applied to me to a considerable degree, or a good part of time OFTEN
- 3. Applied to me very much, or most of the times ALMOST ALWAYS.

### **Clinical examinations**

A clinical examination was carried out using William's graduated probe and no: 23 explorer to record the parameters of gingival index<sup>[14]</sup> (GI), oral hygiene index<sup>[15]</sup> (OHI), probing pocket depth (PPD), and clinical attachment level<sup>[16]</sup> (CAL). All the clinical examinations, group allocation and sample collection, were performed by a single trained examiner (S.J). The analysis of the questionnaire was performed by another examiner (A.M).

The PPD of all the existing teeth was measured at four sites per tooth (mesiobuccal/labial, mid-buccal/ labial, distobuccal/labial, lingual, or palatal) at all the teeth, excluding the third molars, the maximum PPD was recorded.

# Sample collection and determination of the salivary cortisol level

The following instructions were given to the individuals. Two hours before salivary collections, the participants were requested to avoid eating food and drinking beverages. Saliva was collected during morning hours. The sample collection was as per a study by Chiu *et al.*,<sup>[17]</sup> where they emphasized to "spit clear saliva into an Eppendorf tube to minimize the amount of mucous." Following transportation to the laboratory, samples were centrifuged at 3000 rpm for 5 min and stored at  $-20^{\circ}$ C. After thawing, 20 µl of salivary samples were transferred to a sample cup, and cortisol was estimated using a cortisol RP Elecsys kit (Roche Diagnostics, USA) in the cobas e 411 analyzer. Various studies<sup>[18,19]</sup> have reported

that salivary cortisol levels were found to be highly correlated with serum cortisol.

### **Statistical analysis**

The mean and standard deviation of age, PPD, CAL, GI, OHI simplified (OHI-S), and salivary cortisol levels were calculated for each group. Intergroup comparison of salivary cortisol levels, OHI-S, GI and clinical parameters were done using analysis of variance (ANOVA) for normally distributed variables and Kruskal-Wallis test for those not following a normal distribution. Chi-square test was used for finding the association between the clinical parameters. The correlation between clinical parameters, stress, salivary cortisol levels and time served was done using Pearson's rank correlation coefficient. A P < 0.05 was considered statistically significant. The data analyses were performed using a statistical software IBM SPSS 2015 version 20 (IBM, USA).

# RESULTS

The mean age was  $38.56 \pm 10.878$ . The mean OHI-S score ranged from 1.6 to 2.3 in the three groups, which indicated fair hygiene status among the three groups. The ANOVA test revealed that the mean GI score was not statistically significant (P > 0.05) [Table 1]. The ANOVA test results revealed that the mean salivary cortisol level was different in all the three groups, which was statistically significant (P < 0.05). Mean PPD and CAL were highest in Group B,  $6.11 \pm 0.315$  and  $6.11 \pm 1.696$ , respectively.

A positive correlation with a P = 0.093 was obtained between CAL and stress scores. A positive correlation which was statistically significant with a P = 0.011 was obtained between salivary cortisol level and prison time served by the inmates [Table 2]. The multiple comparison test indicated that the mean salivary

### Table 1: Mean and standard deviation of oral hygiene index-simplified, gingival index, salivary cortisol level, and time served by inmates in Groups A, B and C

		Р		
	Group A	Group B	Group C	
OHI-S	2.36±1.02	2.317±1.06	1.69±1.11	0.148
GI	1.51±0.71	1.41±0.72	1.02±0.71	0.119
Salivary cortisol	18.72±2.47	26.08±4.14	9.01±2.67	0.001*
Time served	27.77±30.05	42.74±48.31	12.75±13.64	0.029*

 $^{*}P\!<\!0.05$  - Statistically significant. SD: Standard deviation; OHI-S: Oral hygiene index-simplified; GI: Gingival index

cortisol level increased with an increase in the PPD. The Group B had the highest mean salivary cortisol level compared to the Groups A and C. A strong association was obseved between stress score, cortisol level with CAL and PPD [Table 3].

### DISCUSSION

Cortisol production has a circadian rhythm with levels peaking in the early morning and dropping at night. Levels can rise independently in response to stress. In saliva, the majority of cortisol remained unbound to protein. The normal range of salivary cortisol during morning hours was  $\leq$ 19.1 nmol/L while >19.1 nmol/L was considered to be high.<sup>[17]</sup>

There was no statistically significant difference in the OHI-S and GI between the three groups. It differed from the study conducted by Johannsen *et al.*<sup>[20]</sup> where they found that women with stress-related depression had more plaque accumulation. Since the OHI score in the three groups did not vary, stress may influence periodontal disease by impacting on the physiologic processes.

Individuals in Group B had the highest levels of PPD, CAL, and stress score than the other two groups. CAL and stress showed a positive correlation, but it was not statistically significant. Genco *et al.*<sup>[6]</sup> reported that CAL was significantly associated with increased job and financial strain. Similar cross-sectional studies<sup>[7,21]</sup> have found that individuals with high mean CAL values had higher stress scores than periodontally healthy individuals.

There was a statistically significant association between PPD and stress score. This shows that increase in the PPD was found to occur concomitantly with an increase in stress levels of an individual. Peruzzo et al.[22] in a systematic review reported a positive relationship between stress and periodontal disease. There was no statistically significant association between PPD with anxiety and depression score. On the contrary, in a study conducted by Vettore et al.,[23] frequency of CAL and PPD was found to be significantly associated with higher trait anxiety scores. The assessment of the association between pocket depth and salivary cortisol level showed a strong statistical significance. Hilgert et al.<sup>[24]</sup> and Refulio et al.<sup>[25]</sup> in their cross-sectional study reported that cortisol levels were positively associated with the extent and severity of periodontitis.

Variable	CAL			PPD		Time served		
	Sample size	<b>Correlation coefficient</b>	Р	Correlation coefficient	Р	<b>Correlation coefficient</b>	Р	
Salivary cortisol level	70	0.753	0.001*	0.843	0.001*	0.302	0.011*	
Stress		0.202	0.093	0.057	0.637	0.167	0.166	

Table 2: Pearson's correlation between stress, salivary cortisol, time served, clinical attachment level, and probing pocket depth

\*P<0.05 - Statistically significant. CAL: Clinical attachment level; PPD: Probing pocket depth

Table 3: Association between stres	s, salivary cortisol	, clinical attachment	level, and	probing pocket depth
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Group	Category	C	CAL P PPD			Р		
		Absent (%)	Present (%)		Group A (%)	Group B (%)	Group C (%)	
Stress	Normal	30 (75)	10 (25)	0.031*	25 (64.1)	6 (31.6)	9 (75)	0.025*
	High	15 (50)	15 (50)		14 (35.9)	13 (68.4)	3 (25)	
Salivary cortisol level	Normal	28 (90.3)	3 (9.7)	0.001*	19 (48.7)	0	12 (100)	0.001*
	High	17 (43.6)	22 (56.4)		20 (51.3)	19 (100)	0	

\*P<0.05 - Statistically significant. CAL: Clinical attachment level; PPD: Probing pocket depth

Salivary cortisol levels and stress showed a strong positive correlation, which reveals that when stress increases, salivary cortisol level also increases in a linear pattern. There was a positive correlation between salivary cortisol and PPD, where individuals with higher levels of cortisol had more sites with PPD of 5-7 mm while no significant associations were seen between salivary cortisol level with depression and anxiety score. Various studies<sup>[26,27]</sup> reports that stress score and salivary stress markers were found to be significantly correlated with clinical parameters of periodontal disease and a positive correlation was seen between salivary cortisol levels and PPD and also CAL values. However, in another study,<sup>[28]</sup> no correlation was seen between the cortisol and stress.

Any type of stress, physical or neurogenic causes an immediate and marked increase in the adrenocorticotropin hormone secretion from the anterior pituitary gland followed by a greatly increased secretion of cortisol from the adrenal cortex. Cortisol decreases both the migration of the white blood cells into the inflamed area and the phagocytosis of the damaged cells. It suppresses the immune system by diminishing the IgA and IgG secretions and causing lymphocyte reproduction to be markedly decreased.<sup>[29]</sup>

Merchant *et al.*<sup>[30]</sup> reported that men who had social support were less likely to develop periodontitis compared with men who had low social support. A positive correlation which was statistically significant was obtained between salivary cortisol level and prison time served by the inmates. Salivary

cortisol levels showed a statistical significance in Group B individuals whose time served period was also found to be the highest among the three groups. A significant association was not seen between anxiety, depression with PPD and cortisol levels while a significant association was obtained between stress score, salivary cortisol level, and PPD.

The small sample size and the cross-sectional study design can be taken as limitations of this study. Furthermore, DASS questionnaire is subjective which can produce errors in the data collected. Further longitudinal studies with a larger sample size would be valuable to establish a causal association between psychosocial factors and periodontal health.

# CONCLUSION

It can be summarized that salivary cortisol level can be used as a marker to assess stress. Long-term activation of the stress-response system along with overexposure to cortisol can be detrimental to the overall health of an individual and a risk for periodontitis.

The results of this study indicate that stress and salivary stress markers are correlated with periodontal disease. In addition, immunologic and behavioral changes related to psychological stress may be related to periodontal disease. Future research should further explore the potential mediators of the stress periodontitis relationship and their synergistic and antagonistic interactions.

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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 nonfinancial in this article.

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