

## Evaluation of association between potential stress markers and periodontal health in medical and dental students: A questionnaire-based study

### ABSTRACT

**Aims and Objectives:** Psychological conditions, particularly psychosocial stress, have been implicated as risk indicators for periodontal disease. The aim of the present study was to explore the role of psychosocial stress on periodontium through questionnaire and serum cortisol level.

**Subjects and Methods:** Two hundred medical and dental undergraduates were recruited for the study. Case group included 82 examination going and control group had 79 nonexam going students. Their stress level was evaluated using a standard questionnaire (perceived stress scale). Gingival index, periodontal disease index, bleeding on probing index, serum cortisol level, and serum alpha-amylase level were also measured.

**Statistical Analysis Used:** Bivariate correlations and multiple regression tests were done.

**Results:** A positive correlation was found among stress scores, salivary cortisol, alpha-amylase, and periodontal disease measures.

**Conclusion:** Periodontitis can be related to immunologic changes related to psychological states

**Keywords:** Cortisol, examination, periodontitis, stress

### INTRODUCTION

Periodontitis is an inflammatory disease involving the destruction of the investing tissues around the teeth, resulting in loss of tooth support, ultimately tooth loss.

The etiology of periodontal disease involves numerous risk factors such as age, smoking, specific infections, uncontrolled diabetes, psychosomatic conditions such as anxiety and psychosocial stress.<sup>[1-3]</sup>

Stress is a state of physiological or psychological strain caused by adverse stimuli, physical, mental, emotional, internal or external that tend to disturb the functioning of an organism.<sup>[4]</sup> Researchers have reported an association between psychological stress and gingival inflammation and periodontitis.<sup>[5-9]</sup>

Gingivitis is mild, reversible form of periodontal disease characterized by gingival inflammation without attachment

loss and detected clinically by bleeding on probing (BOP). Untreated gingivitis may evolve into periodontitis, a chronic inflammatory state resulting in periodontal attachment loss.<sup>[10]</sup> Clinical indicators of periodontitis include probing pocket depth, gingival recession, clinical attachment level, and radiographic loss of alveolar bone.<sup>[11]</sup>

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**Received:** 26 December 2019, **Revised:** 13 February 2020, **Accepted:** 12 March 2021, **Published:** 20 April 2022

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**How to cite this article:** Agarwal P, Bhattacharya HS, Rastogi P, Agarwal MC, Agarwal A. Evaluation of association between potential stress markers and periodontal health in medical and dental students: A questionnaire-based study. *Natl J Maxillofac Surg* 2022;13:90-4.

Access this article online	
<b>Website:</b> www.njms.in	<b>Quick Response Code</b> 
<b>DOI:</b> 10.4103/njms.NJMS_101_19	

Chronic activation of the hypothalamic–pituitary–adrenal axis may influence the initiation and progression of periodontitis showing dysregulation of circulating cortisols and other glucocorticoids that affect immune function.<sup>[12]</sup> Green *et al.* reported higher incidence of periodontal diseases in those experiencing stressed life events and a particularly strong correlation between stressors and periodontal disease.<sup>[13]</sup>

In addition to this, occupational and academic stress may be associated with progression of periodontal disease. In a small study over the Nigerian students, those undergoing academic examinations had more periodontal inflammation than controls.<sup>[14]</sup>

A systematic review<sup>[15]</sup> of case control, cross-sectional and prospective studies examining psychological stress and periodontal disease indicated that 57.1% of the studies reported a positive correlation between psychological stress and periodontal disease and 14.2% did not.

The present study extends the research on chronic stress, depression and periodontal disease by measuring behaviors, psychological variables, and salivary stress markers such as alpha-amylase and cortisol levels to explore the behavioral and immunologic correlates of periodontal parameters.

## SUBJECTS AND METHODS

### Study population

The study was conducted in Rohilkhand Medical College and Hospital and Institute of Dental Sciences (IDS), Bareilly.

A total of 200 dental and medical undergraduates were included. Out of these, 100 undergraduates were undergoing professional examination and 100 were in nonexam giving group.

The subjects were in the age range of 18–21 years with minimum 20 teeth excluding third molars. Participants on antibiotics, steroids, chemotherapeutic agents, or antipsychotic drug therapy and with immunosuppressive diseases were excluded [Table 1].

The study protocol was approved by the ethical committee of IDS (Reference Number: IEC/IDS/102/2019) dated 6<sup>th</sup> January 2022 and informed written consent was taken from all the participants.

### Clinical parameters and health survey

#### The clinical parameters

1. Gingival index (GI), Loe and Silness 1963,<sup>[16]</sup> records qualitative changes in gingiva

2. Periodontal disease index (PDI), Ramfjord 1967,<sup>[17]</sup> assess the prevalence and severity of gingivitis and periodontitis within the individual dentitions and in population groups
3. BOP index.

These were measured by a single examiner and the stress scale ratings by the other.

The health and oral hygiene survey included questions about age, family history of periodontal disease, smoking, and frequency of brushing and flossing. Participants also indicated whether they neglected oral hygiene during periods of stress or depression.

### Psychological evaluation

Participants from both, examination giving and nonexam-giving group answered a questionnaire pertaining to the level of stress that they perceive<sup>[18]</sup> perceived stress scale, [Table 2]. According to the interpretation of the scores [Table 3], participants from the exam giving group who scored above 20 were included in case group. Similarly, participants from nonexam-giving group who scored < 11 were included in the control group. This made a case group of 82 participants and control group of 79 participants.

### Saliva sample

Saliva was collected by passive drool through a 1-inch straw into a vial. Samples were drawn between 9 am and 10 am to avoid circadian rhythm changes.<sup>[19]</sup> Refrigerate samples within 30 min, and freeze at or below –20°C within 4 h after collection. On day of assay, thaw completely, vortex, and centrifuge at 1500 × g (@3000 rpm) for 15 min. Samples should be at room temperature before adding to assay plate. Salivary cortisol and alpha-amylase were assayed using kits (Salimetrics salivary cortisol assay kit).

### Statistical analyses

Mean stress scores, salivary stress markers, and clinical

**Table 1: Description of sample (n=161)**

Variable	Values
Age (years), mean (SD)	18.55 (1.43)
Sex (males: females)	45:55
History of smoking (%)	36
History of alcohol (%)	46
Neglects brushing when stressed (%)	34.2
Tooth brushing (number of times/day; mean) (%)	
Once	27.3
Twice	66.5
Thrice	6.2
Stress score, mean (SD)	
Control	8.75 (1.70)
Case	28.40 (1.85)

SD: Standard deviation

**Table 2: Perceived Stress Scale**

Question	Rating	Score
1. How often have you been upset because of something that happened unexpectedly?		
2. How often have you felt that you were unable to control the important things in your life?		
3. How often have you felt nervous and "stressed"?		
4. How often have you felt confident about your ability to handle your personal problems?		
5. How often have you felt that things were going your way?		
6. How often have you found that you could not cope with all the things that you had to do?		
7. How often have you been able to control irritations in your life?		
8. How often have you felt that you were on top of things?		
9. How often have you been angered because of things that were outside of your control?		
10. How often have you felt difficulties were piling up so high that you could not overcome them?		

Students were asked to fill it in perspective of their last 1 month of their professional exams

parameters were calculated for both case and control groups [Table 4]. Bivariate correlations were done among psychosocial variables, salivary stress markers, and periodontal disease measures [Tables 5 and 6]. Correlation was significant at 0.01 level and it was one tailed. Multiple regressions were used to relate periodontal disease measures with psychosocial variables, stress scores, and salivary stress markers [Tables 7-9].

**RESULTS**

Of the total 161 participants, 36% were smokers and 46% had a history of alcoholism. Most of the participants brushed their teeth twice daily (about 66.5%) and 34.2% gave a history of no brushing during stress [Table 1]. The GI and PDI means (standard deviation) were 1.75 (0.42) and 0.92 (0.43) respectively for control group and 2.69 (0.21) and 1.79 (0.13) respectively for case group. A positive correlation existed among stress scores, salivary cortisol and alpha-amylase and the periodontal disease measures. Stress score was significantly correlated with all three periodontal disease measures, i.e., GI, PDI, and BOP index [Table 6]. However, there was negative correlation between stress score and brushing frequency [Table 5].

**DISCUSSION**

The present study showed direct correlation between periodontal disease measures, stress scores, and salivary stress markers. The results were consistent with previous studies suggesting the association of periodontal disease with stress.<sup>[3,12]</sup>

In terms of behavior, the study did not show any significant correlation between brushing frequency and stress scores and markers. This was in contradiction to the previous studies.<sup>[20,21]</sup> Thus, the relationships among stress, oral hygiene, and markers of periodontal disease were unclear. The effect of oral hygiene may not be apparent because of

**Table 3: Interpretation**

Total score	PSS level	Health concern level
0-7	Much lower than average	Very low
8-11	Slightly lower than average	Low

PSS: Perceived Stress Scale

**Table 4: Mean values of salivary stress markers, disease parameters in case and control group**

Groups	Mean (SD)				
	Cortisol	Amylase	GI	PI	BOP
Case	1.2302 (0.3105)	142.5146 (5.9356)	2.6951 (0.2113)	1.7976 (0.1296)	95.5427 (1.9353)
Control	0.4340 (0.3140)	110.0722 (12.7141)	1.7582 (0.4177)	0.9228 (0.4332)	78.0987 (14.7785)
Total	0.8395 (0.5063)	126.5957 (19.0084)	2.2354 (0.5731)	1.3683 (0.5408)	86.9832 (13.5974)

SD: Standard deviation, GI: Gingival index, PI: Periodontal index, BOP: Bleeding on probing

**Table 5: Bivariate correlations among psychosocial variables and salivary stress scores and markers**

Variables	Stress score	Cortisol	Amylase
Brushing	-0.022	0.004	0.007
No brushing	-0.059	0.014	-0.027

**Table 6: Bivariate correlations among periodontal disease measures and stress scores and salivary markers**

Periodontal measures	Stress score	Cortisol	Amylase
GI	0.808**	0.951**	0.974**
PDI	0.799**	0.931**	0.976**
BOP	0.632**	0.827**	0.881**

\*\*This data shows a positive correlation between periodontal disease measures and salivary stress markers. GI: Gingival index, PI: Periodontal index, BOP: Bleeding on probing

socioeconomic class of sample selected and maintenance and awareness of hygiene maintenance.

A positive relationship exists among depression scores, salivary cortisol, and alpha-amylase and the indices of periodontal disease. This is likely because of altered immune responses that facilitate increased colonization

**Table 7: Standardized coefficients of linear regression analysis relating stress and stress markers with periodontal index ( $R^2=0.954$ )**

Variable	$\beta$	$t$	Significance
Sex	-0.012	-0.555	0.580
Smoking	-0.012	-0.350	0.727
Alcohol	0.024	0.801	0.424
No brush	-0.013	-0.729	0.467
Brush	0.019	1.110	0.269
Stress score	-0.103	-3.075	0.002
Cortisol	-0.115	-1.824	0.070
Amylase	1.172	15.887	0.000

**Table 8: Standardized coefficients of linear regression analysis relating stress and stress markers with gingival index ( $R^2=0.952$ )**

Variables	$\beta$	$t$	Significance
Sex	-0.010	-0.421	0.674
Smoking	-0.014	-0.409	0.683
Alcohol	-0.022	-0.693	0.489
No brush	-0.016	-0.870	0.386
Brush	-0.009	-0.522	0.602
Stress score	-0.021	-0.619	0.537
Cortisol	0.213	3.299	0.001
Amylase	0.785	10.396	0.000

**Table 9: Standardized coefficients of linear regression analysis relating stress and stress markers with bleeding on probing ( $R^2=0.827$ )**

Variables	$\beta$	$t$	Significance
Sex	-0.014	-0.332	0.741
Smoking	0.011	0.169	0.866
Alcohol	0.043	0.723	0.471
No brush	-0.011	-0.306	0.760
Brush	0.030	0.893	0.373
Stress scores	-0.453	-6.951	0.000
Cortisol	-0.409	-3.338	0.001
Amylase	1.655	11.580	0.000

by pathogenic bacteria and the symptoms of periodontal disease.<sup>[20,22,23]</sup>

The immune response does not operate autonomously but in close cooperation with the neuroendocrine systems. When the body is in stress, the glucocorticoids released through the activation of the hypothalamus–pituitary–adrenal axis seem to be important, due to their ability to regulate the recruitment of immune cells into inflamed tissues and to skew the Th1/Th2 balance toward a Th2-dominant response, thereby leading to the progression of periodontal disease.<sup>[24]</sup> In the presence of stress hormone, collagen production is shown to be decreased due to increase in amounts of glucocorticoids.<sup>[25]</sup> Deinzer *et al.* reported that academic stress can lead to gingival inflammation with increased

crevicular interleukin-1b and diminished quality of oral hygiene. Furthermore, stress modifies the salivary pH and its chemical composition like IgA secretion, thus contributing to gingival and periodontal inflammation.<sup>[7]</sup>

Multiple regression analysis showed that stress scores and salivary amylase were highly significant predictors of PDI and cortisol was marginally significant predictor after controlling for sex, smoking, and alcohol history and brushing frequency [Table 7].

Analysis also depicted cortisol and amylase as highly significant predictors of GI [Table 8], While for BOP, stress scores, cortisol, and amylase emerged to be highly significant predictors after controlling for sex, smoking, alcohol, and brushing frequency [Table 9].

Salivary cortisol showed positive correlation with periodontal disease measures, but in regression models, it was seen that cortisol was a marginally significant predictor of PDI. Although this finding may seem counterintuitive, it is consistent with recent research on stress that distinguishes between acute stress and the chronic, debilitating negative effect that is more likely to be associated with depression and flattened cortisol patterns.<sup>[26]</sup> Subjective distress, feeling out of control, and traumatic or physically threatening stress are associated with lower morning levels and suppressed diurnal variability of cortisol.<sup>[26]</sup>

Patients experiencing such changes in cortisol may eventually have immune effects that result in periodontitis.

## CONCLUSION

The results showed positive correlations among stress, salivary stress markers and PDI, independent of dental hygiene. Therefore, it can be concluded that periodontitis can be related to immunologic changes related to psychological states. Further, cortisol seems to have different associations with periodontal outcomes in regression models involving stress.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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