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ORIGINAL ARTICLE

Association of stress, salivary alpha-amylase and periodontal conditions in patients visiting a tertiary care institution

Fizza Tariq [1]✉, Qamar Aziz [1], Saba Abrar [1], Sobia Khan Nabeel[1], Tayyaba Kazmi [2], Syed Adnan Ahmed[1]

1: Department of Physiology, Baqai Medical University, Karachi, Pakistan
2: Department of Anatomy, Baqai Medical University, Karachi, Pakistan

Cell #: +92-341-3257122
Email✉: fizzatariq@baqai.edu.pk

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ABSTRACT

Objective: To assess the association between psychological stress, salivary alpha-amylase (sAA) levels, and periodontal diseases (gingivitis and periodontitis).

Methods: This unmatched case-control study was conducted at Baqai Medical University and its affiliated institutes, Karachi, Pakistan, in 2023. A total of 88 participants aged 18-45 years were enrolled, comprising 28 healthy controls, 30 patients with gingivitis, and 30 with periodontitis. Periodontal status was assessed using the Community Periodontal Index of Treatment Needs (CPITN), periodontal pocket depth, and clinical attachment level. Perceived stress was evaluated using the 10-item Perceived Stress Scale (PSS-10), while salivary alpha-amylase levels were quantified by enzyme-linked immunosorbent assay (ELISA). Statistical analyses included Chi-square test, one-way ANOVA, Tukey's HSD test and Spearman rank correlation.

Results: The study groups were comparable in age and gender ($p>0.05$). Moderate-to-high perceived stress was significantly more common among patients with periodontitis (86.7%) and gingivitis (86.6%) than controls (35.7%) ($p<0.01$). Mean salivary alpha-amylase levels were significantly higher in the periodontitis ($6.8\pm0.5\,\mu$/ml) and gingivitis ($5.2\pm1.2\,\mu$/ml) groups than in controls ($2.4\pm0.6\,\mu$/ml) ($p<0.01$). Pairwise comparisons showed significant differences in sAA levels among all three groups ($p<0.01$). Salivary alpha-amylase demonstrated a moderate positive correlation with perceived stress ($r=0.428$, $p<0.01$), whereas no significant associations were observed with age or gender.

Conclusion: Patients with gingivitis and periodontitis exhibited significantly higher perceived stress and salivary alpha-amylase levels than healthy controls. Salivary alpha-amylase showed a positive association with psychological stress, supporting its potential role as a non-invasive biomarker of stress in periodontal disease.

Keywords: Gingivitis (MeSH); Periodontitis (MeSH); Periodontal Disease (MeSH); alpha-Amylases (MeSH); Salivary alpha-Amylase (MeSH); Stress Disorder, Traumatic (MeSH); Stress, Psychological (MeSH); Biomarkers (MeSH); Adult (MeSH); Oral Health (MeSH).

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INTRODUCTION

Periodontal disease is a major global oral health problem, affecting approximately 11% of the world's population.[1] Gingivitis and periodontitis are multifactorial inflammatory disorders involving the supporting tissues of the teeth, including the gingiva, periodontal ligament, cementum and alveolar bone.[2,3] Gingivitis is a plaque-induced inflammatory condition confined to the gingival tissues. If left untreated, it may progress to periodontitis, a chronic, irreversible inflammatory disease characterized by progressive destruction of the periodontal ligament, cementum and alveolar bone, ultimately leading to tooth loss.[4] Dental plaque forms a pathogenic biofilm that promotes bacterial proliferation, persistent inflammation, periodontal pocket formation, and alveolar bone resorption, which constitute the key pathogenic mechanisms underlying periodontitis.[4-6]

Various contributing risk factors have been recognized as associated with periodontal disease including age, genetics, stress, hormonal imbalance, diabetes mellitus, poor oral hygiene, poor nutrition, smoking, alcohol and certain medications.[7]

Stress is a physiological and psychological response to stimuli that disrupts homeostasis and increases disease risk.[8] Several research studies have reported the significant association between stress and higher risk of periodontal disease.[8-10] Globally, stress has become widespread concern in today's era. According to American Psychological Society approximately 20% of American population suffer from high degree stress.[9] Stress activates the SAM and HPA pathways, increasing catecholamines and cortisol to help the body adapt. However, chronic stress disrupts these systems, weakens immune responses and promotes inflammation, contributing to the development and progression of periodontal disease.[11-13]

Previous studies have demonstrated that psychological stress is associated with elevated serum and salivary cortisol levels, which correlate positively with the presence and severity of periodontal disease.[14] Stress also activates the sympatho-adrenal medullary (SAM) axis, increasing catecholamine release and stimulating salivary alpha-amylase secretion.[12]

Consequently, sAA has emerged as a non-invasive biomarker of stress, with elevated levels reported in patients with periodontitis.[12,15] However, evidence regarding its association with gingivitis remains limited. Nouri P, et al. also reported a positive association between psychological stress, elevated salivary alpha-amylase and cortisol levels and moderate-to-severe periodontitis.[16] Saliva sampling offers a rapid, non-invasive, and painless approach for biomarker assessment, making sAA a promising indicator of stress.[17]

Despite growing evidence linking psychological stress to periodontal disease, limited data are available from Pakistan, particularly regarding the association of perceived stress and salivary alpha-amylase with both gingivitis and periodontitis. Therefore, the present study was planned to assess the relationship between psychological stress, salivary alpha-amylase levels, and periodontal disease among adults in Karachi, Pakistan. The findings may improve understanding of the role of stress in periodontal disease progression and support the use of stress assessment as an adjunct in periodontal risk evaluation and management

METHODS

This unmatched case-control study was conducted at Baqai Medical University and its affiliated institutes in Karachi, Pakistan, during 2022. The study protocol was approved by the Ethical Committee of Baqai Medical University (Reference No. BMU-EC/03-2021; dated: May 03, 2021) and the Board of Advanced Studies and Research (BAS&R). Written informed consent was obtained from all participants before enrollment.

The sample size was calculated using OpenEpi for an unmatched case-control study based on Fleiss' formula with continuity correction. Assuming the specified confidence level, study power, odds ratio, and expected exposure among cases and controls, the required sample size was estimated to be 88 participants.

Participants of either sex, aged 18-45 years, with more than 10 natural teeth were eligible for inclusion. Patients attending the dental outpatient department for routine dental examination, scaling, or complaints of plaque or calculus deposition, gingival bleeding, gingival swelling, or tooth mobility were considered for recruitment. A total of 88 individuals aged 18-45 years were enrolled, including 28 healthy controls and 60 patients with periodontal disease (30 with gingivitis and 30 with periodontitis). Individuals with systemic diseases including diabetes mellitus, hypertension, myocardial infarction, rheumatoid arthritis, renal disease, allergies, or other chronic medical conditions were excluded. Pregnant or lactating women, smokers, betel nut chewers, individuals receiving hormonal or antipsychotic therapy and those with a history of psychiatric or mental illness were also excluded.

Demographic and clinical information, including age, sex, educational status, socioeconomic status, anthropometric measurements, dental history, and smoking history, were collected using a structured questionnaire. Periodontal status was assessed by a certified periodontist using the Community Periodontal Index of Treatment Needs (CPITN), periodontal pocket depth (PPD), and clinical attachment level (CAL) measured with a calibrated periodontal probe.

Psychological stress was assessed using the validated 10-item Perceived Stress Scale (PSS-10).[18] Participants were categorized as having low (0-13), moderate (14-26), or high (27-40) perceived stress according to the total PSS-10 score.

Unstimulated whole saliva was collected before periodontal examination to avoid contamination from gingival bleeding. Participants were instructed to refrain from eating or drinking for at least one hour before sample collection and to rinse their mouth with water for 15 seconds immediately beforehand. Saliva samples were collected into sterile labeled tubes, refrigerated at 4°C, centrifuged at 4,000 × g for 20 minutes at 4°C and the supernatant was aliquoted and stored at -80°C until analysis.

Salivary alpha-amylase concentrations were determined using a commercially available ELISA kit (Euroimmun, Germany) according to the manufacturer's instructions. Absorbance was measured at 450 nm using the SkanIt Microplate Reader Software RE (Version 5.0.0.42).

Data were analyzed using SPSS version 23.0 (IBM Corp., Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation, while categorical variables were presented as frequencies and percentages. Comparisons between groups were performed using the independent-samples t-test, one-way ANOVA with Tukey's post hoc test, and the Chi-square test, as appropriate. Spearman's rank correlation was used to assess the relationship between salivary alpha-amylase levels and perceived stress, while binary logistic regression analysis was performed to identify factors associated with periodontal disease. A p-value <0.05 was considered statistically significant.

RESULTS

The demographic characteristics of the study participants are presented in Table I. A total of 88 participants were enrolled, including 28 healthy controls, 30 patients with periodontitis, and 30 with gingivitis. The mean age was comparable among the Control (30.4±6.81 years), Periodontitis (33.1±6.61 years), and Gingivitis (30.1±6.52 years) groups, with no statistically significant difference ($p=0.70$). Similarly, gender distribution was comparable across the groups, with females comprising 50.0%, 56.7%, and 53.3% of the Control, Periodontitis, and Gingivitis groups, respectively ($p=0.87$). These results indicate that the study groups were well-matched for age and gender, providing a consistent baseline for further clinical comparisons.

Perceived stress differed significantly among the study groups ($p<0.01$) (Table II). Moderate-to-high perceived stress was reported by 86.7% of patients with periodontitis and 86.6% of those with gingivitis, compared with 35.7% of healthy controls. High perceived stress was also more frequent among patients with gingivitis (33.3%) and periodontitis (30.0%) than

Table I: Demographic characteristics of study groups

Characteristics		Control (n=28)	Periodontitis (n=30)	Gingivitis (n=30)	p-value
Age Group	18 - 30 years	15 (53.6%)	11 (36.7%)	18 (60.0%)	0.17
	31 - 45 years	13 (46.4%)	19 (63.3%)	12 (40.0%)	
	Mean±SD	30.4±6.81	33.1±6.61	30.1±6.52	
Gender	Male	14 (50.0%)	13 (43.3%)	14 (46.7%)	0.87
	Female	14 (50.0%)	17 (56.7%)	16 (53.3%)	

Note: All values are presented as percentage (%);*p<0.05 was considered statistically significant.

Table II: Perceived Stress Scale score among the participants of the study groups

Perceived Stress Scale Score	Control (n=28)	Periodontitis (n=30)	Gingivitis (n=30)	p-value
Low Stress	18 (64.3%)	4 (13.3%)	4 (13.3%)	<0.01*
Moderate Stress	8 (28.6%)	17 (56.7%)	16 (53.3%)	
High Perceived Stress	2 (7.1%)	9 (30.0%)	10 (33.3%)	

Note: All values are presented as percentage (%);*p < 0.05 was considered statistically significant

controls (7.1%). No significant association was observed between perceived stress and age or gender (p>0.05).

Levels of sAA were measured in both groups, the results showed that the mean concentration of sAA was 2.4±0.6 μ/ml in control group, 6.8±0.5 μ/ml in the periodontitis and 5.2±1.2 μ/ml in the gingivitis group. The one-way ANOVA demonstrated a statistically significant difference in the mean sAA levels among the study groups (p<0.01). With respect to gender and age groups, statistically insignificant mean difference in sAA was noted across study groups (p > 0.05 for age and gender).

Post hoc Tukey's HSD test statistically significant difference in sAA levels was noted between following groups: control vs. periodontitis (p<0.01), control vs. gingivitis (p<0.01) and periodontitis vs. gingivitis (p<0.01).

Results of Spearman rank correlation test displayed a moderately (42.8%) positive correlation between sAA and PSS. Whereas, R2 exhibited 17.5% variation in PSS and attributed to variations in sAA. Furthermore, no significant association was found between sAA, PSS, gender and age groups.

DISCUSSION

The present study demonstrated a significant association between perceived psychological stress and periodontal disease. Patients with gingivitis and periodontitis exhibited significantly higher perceived stress than healthy controls, with moderate-to-high stress observed in approximately 87% of both periodontal disease groups. Although the overall prevalence of psychological stress was comparable between the gingivitis and periodontitis groups, high perceived stress was slightly more frequent among patients with gingivitis. In contrast, healthy individuals predominantly reported low perceived stress. These findings suggest that psychological stress may play an important role in the development and progression of periodontal disease.

Several studies have identified psychological stress and related conditions, including anxiety and depression, as important risk factors for the development and progression of periodontal disease.[19] Stress-induced activation of the sympathetic nervous system increases salivary alpha-amylase (sAA) secretion, making it a useful surrogate biomarker of sympathetic activity.[15,17,20] However, exact prevalence of periodontal diseases (gingivitis and periodontitis) among individuals experiencing stress or contrariwise remains uncertain. Consequently, estimation of sAA may provide a valuable diagnostic tool for measuring the impact of stress on periodontal health, to recognize population at risk of stress-induced periodontal disorders.[21] Therefore, the present study evaluated perceived stress and salivary alpha-amylase levels in patients with gingivitis and periodontitis compared with healthy controls to further explore the role of stress in periodontal disease.

The study groups were comparable with respect to age and gender. The present study demonstrated a significant association between perceived stress and periodontal disease, with patients having gingivitis and periodontitis exhibiting substantially higher stress levels than healthy controls. Moderate-to-high perceived stress predominated in both periodontal disease groups, whereas most healthy participants reported low perceived stress. These findings support the role of psychological stress as a potential contributor to the development and progression of periodontal disease.

The study groups were comparable with respect to age and gender. The present study demonstrated a significant association between perceived stress and periodontal disease, with patients having gingivitis and periodontitis exhibiting substantially higher stress levels than healthy controls. Moderate-to-high perceived stress predominated in both periodon-tal disease groups, while high perceived stress was slightly more frequent among patients with gingivitis than those with periodontitis (33.3% vs. 30.0%). In contrast, most healthy participants reported low perceived stress. These findings support the role of psychological stress as a potential contributor to the development and progression of periodontal disease. Similar findings were reported by Lee YH, et al., who demonstrated a positive association between psychological stress and periodontal disease.[22] However, unlike the present study, they observed a higher frequency of high perceived stress among patients with periodontitis than gingivitis (44% vs. 41%). This discrepancy may be attributable to differences in study populations, assessment methods, or the relatively small sample size of the present study.

Study of Agarwal and colleagues was in line with the present study results who also reported a significant association of stress score with periodontal diseases ascribed to stress-induced immunological alterations.[23] Another study of Carolina and colleagues observed high stress level only in patients with severe periodontal conditions and found no significant difference in stress levels between periodontitis and gingivitis groups when measured with PPS-14.[24]

Salivary biomarkers provide an objective measure of the physiological stress response and are considered more reliable than subjective stress assessment questionnaires.[21] In the present study, sAA levels were significantly higher in patients with gingivitis and periodontitis than in healthy controls, with the highest levels observed in the periodontitis group. Furthermore, sAA levels differed significantly among all three groups ($p<0.01$), and regression analysis demonstrated that elevated sAA levels were associated with an increased risk of both gingivitis and periodontitis. These findings support a positive association between salivary alpha-amylase and periodontal disease. Similar observations have been reported in previous studies, which demonstrated persistently elevated sAA levels in patients with periodontal disease and a positive correlation with disease severity, likely reflecting enhanced sympathetic activation and inflammatory responses.[17,25] In contrast, Develioglu H, et al., reported a significant association between psychological stress, salivary cortisol, and chronic periodontitis, but found no significant association with salivary alpha-amylase.[15] The discrepancy may be related to differences in study design, study population, disease characteristics, or methods used for stress assessment and biomarker measurement.

The present study demonstrated a significant positive correlation between perceived stress and salivary alpha-amylase levels, indicating that higher psychological stress is associated with greater sympathetic activation. These findings are consistent with a systematic review by Decker A, et al., which reported a positive association between psychological stress, salivary stress biomarkers, and periodontal disease severity.[26] Similarly, Coelho JM, et al., found that psychological stress was significantly associated with an increased risk of periodontitis.[9] In contrast, Shende A, et al., observed no significant association between stress and periodontal disease in a pilot study and emphasized the need for larger longitudinal studies to clarify this relationship.[27]

No significant differences in perceived stress or salivary alpha-amylase levels were observed across age or gender groups, although gingivitis was more common among younger participants (18-30 years) and periodontitis among older participants (31-45 years). Similar findings regarding the lack of gender differences have been reported by Develioglu H, et al.[15] In contrast, Shende A, et al. suggested that women are more susceptible to psychological stress and may therefore have a higher risk of periodontal disease.[9] Previous studies have also reported an increased prevalence of periodontitis with advancing age, which may be attributable to cumulative psychological stress, adverse life events, and poorer coping mechanisms.[9,28,29]

The findings of the present study support the potential utility of salivary alpha-amylase as a non-invasive biomarker of psychological stress in patients with periodontal disease. Incorporating stress assessment and salivary biomarkers into periodontal evaluation may facilitate early identification of high-risk individuals and contribute to more comprehensive patient management. However, larger prospective longitudinal studies are required to validate these findings and establish the diagnostic and prognostic value of salivary alpha-amylase in periodontal disease.

Limitations of the study

Psychological stress was assessed using a self-reported questionnaire without formal psychiatric or psychological evaluation, which may have affected the accuracy of stress assessment. In addition, the relatively small sample size and single-center design may limit the generalizability of the findings. Further multicenter studies with larger sample sizes and specialist-based psychological assessment are recommended.

CONCLUSION

This is the first study from Karachi, Pakistan, to evaluate the association of psychological stress and salivary alpha-amylase with both gingivitis and periodontitis. Patients with periodontal disease exhibited significantly higher perceived stress and salivary alpha-amylase levels than healthy controls, supporting a positive association between psychological stress and periodontal disease. These findings suggest that salivary alpha-amylase may serve as a useful non-invasive biomarker of stress and highlight the importance of incorporating stress assessment into comprehensive periodontal care. Dentists should consider appropriate referral of patients with significant psychological stress to mental health professionals, while individuals experiencing stress should be encouraged to undergo regular periodontal evaluation and preventive dental care. Further multicenter prospective and interventional studies are warranted to determine whether effective stress management improves periodontal health and to establish the diagnostic and prognostic utility of salivary alpha-amylase in periodontal disease.

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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

FT: Conception and study design, acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

QA & SA: Conception and study design, critical review, approval of the final version to be published

SKN: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

TK & AA: Acquisition, analysis and interpretation of data, critical review, approval of the final version to be published

Author agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DISCLAIMER

This research article is the original work of the Author and a part of M. Phil Thesis.
It has not been presented or published in a conference, or published in an abstract book before.

KMUJ web address: www.kmuj.kmu.edu.pk
Email address: kmuj@kmu.edu.pk