wjpmr, 2025, 11(10), 232-235

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

SJIF Impact Factor: 6.842

Research Article

ISSN (O): 2455-3301

ISSN (P): 3051-2557

A COMPARATIVE STUDY ON ASSESSING THE INFLUENCE OF STRESS ON SALIVARY ALPHA AMYLASE IN FIRST YEAR DENTAL COLLEGE STUDENTS BEFORE AND AFTER INTERNAL EXAMINATION

Nelson Aruldoss*, Elakkiya Pichaimani, Sharon Gladsie

Rajas Dental College and Hospital, Tirunelveli – 627105, India.

***Corresponding Author: Dr. Nelson Aruldoss**

Rajas Dental College and Hospital, Tirunelveli - 627105, India.

DOI: https://doi.org/10.5281/zenodo.17223893

Article Received on 01/08/2025 **Article Revised on 22/08/2025** **Article Accepted on 12/09/2025**

ABSTRACT

Stress is an integral component of human life and is often regarded as a defining characteristic of modern living. Depending on individual perception and coping mechanisms, it can act as a source of motivation or a precursor to psychological distress. Despite extensive research, a reliable and non-invasive biochemical marker for accurately assessing stress intensity has not yet been firmly established. In recent years, saliva has emerged as a promising medium for identifying biomarkers associated with various physiological and behavioral responses. Salivary secretion is primarily regulated by the sympathetic nervous system via activation of the sympatho-adreno-medullary (SAM) axis, which is closely linked to acute stress. Activation of this axis influences the secretion of salivary α-amylase, with its levels fluctuating in accordance with stress intensity. Consequently, salivary α-amylase is increasingly recognized as a potential non-invasive biomarker for evaluating acute stress in humans. In the present study, stress level and salivary amylase activity was measured an hour before and after among 30 students from Bachelor of Dental Surgery (BDS) first years. The level of the salivary amylase was found to be significantly increased an hour before exam and significantly reduced an hour after the exam. Elevated salivary α-amylase activity observed among students may reflect increased physiological stress levels in this population. Moreover, data analysis demonstrated a strong positive correlation between stress scores and salivary α-amylase activity, reinforcing the potential of this enzyme as a reliable biochemical indicator for assessing stress intensity.

KEYWORDS: Stress, Salivary a amylase.

INTRODUCTION

Stress has become an omnipresent feature of contemporary society, with its intensity varying considerably across human populations. Depending on the nature of its stimulus, stress may manifest as **eustress** (beneficial, motivating) or **distress** (harmful, debilitating) (Yamaguchi et al, 2004). While a moderate level of stress can be energizing, protective, and conducive to improved performance, excessive or chronic stress is associated with severe health consequences, including clinical depression, immunosuppression, cancer, and cardiovascular disease. This has prompted significant scientific interest in developing reliable, non-invasive biochemical markers for stress assessment (George S et al 2018).

Previous studies have reported substantial variability in salivary biomarkers—such as salivary cortisol (sC), salivary α-amylase (sAA), and salivary chromogranin A (sCgA)—under different stress conditions (Hellhammer et al, 2009; Ali N, Nater et al.). However, owing to the multifaceted nature of stress, a single definitive biochemical parameter for quantitative non-invasive stress measurement remains elusive. Evidence suggests that sAA levels rise rapidly in response to both physiological and psychological stress, making it a promising candidate biomarker. Stress activates two principal neuroendocrine pathways: the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adreno-medullary (SAM) axis. Activation of the HPA axis stimulates cortisol secretion, while SAM axis activation triggers catecholamine release and salivary α-amylase secretion, though these responses exhibit distinct temporal dynamics (Ali SQ et al; Chatterton et al, 1996; Nater et al, 2005; Stegeren et al, 2006).. For instance, serum norepinephrine levels typically rise 20–30 minutes after stress onset, whereas salivary α-amylase activity increases within minutes, providing a faster index of stress (Yamaguchi et al, 2004, Shkulaku, 2015).

Studies have further demonstrated that students undergoing examination-related psychological stress exhibit a marked rise in sAA activity (Chatterton et al, 1996; Mckay et al, 2010, George S et al 2018). First year

students in a professional college can be considered as a vulnerable group because they are in a transition stage from the school education to the professional education and find it difficult to cope up with the new system. The highest ranking stressor for first-year students was fear of failing course or year. Additionally, parental pressure, fear of criticism also plays a vital role in increased stress. This study is done to compare the stress levels by performing non-invasive assessment i.e., Salivary Alpha amylase test in First year students before and after their internal exams.

The present study is the maiden attempts to explore the effect of sAA activity and stress levels of the students before and after the examination in a single day. Further the study will also validate if the level of salivary activity can be taken as an indices of stress level.

MATERIALS AND METHODS

Sample collection

This study includes collection of samples from 30 students studying first year BDS in Rajas Dental College and Hospital, Tirunelveli District, Tamil Nadu, India, whose age ranges between 17-20 year especially with no systemic diseases (Students with any pre-existing health conditions were excluded from participation to eliminate potential confounding factors). We opted the day of First internal examination for sample collection and the time duration was one day.

This study also includes a self-assessment questionnaire to be filled up by the student participants. Prior to completing the self-assessment questionnaire, the participants were provided with a detailed explanation of the study's aims and objectives and informed consent was obtained from each individual. The stress questionnaire (BFEC Stress questionnaire, http://bfec.kenyon.edu/Healthy_Kenyon/stress_psymptoms .pdf) used in this study was easy to understand and self-explanatory. It consisted a total 50 questions, with five options for marking i.e. Never, Seldom, Sometime, Often and Regular depending upon the frequency of occurrence. The category of options was assigned as a number 0, 1, 2, 3, 4 and 5 for Never, Seldom, Sometime, Often and Regular respectively. Stress level of the subject was computed by adding the total scores. The scores and its indications are calculated as follow

Total scores of an individual according to the self-assessment questionnaire	Stress level indication
0-20	No stress
21-45	Stress below average
46-70	Average stress
71-90	Stress above average
>91	Highly stressed

From the selected 30 students' participants who were involved in the study, two different sets of saliva samples were collected at particular time period of one hour before approaching the examination and one hour after completing the examination. Both the scores of the self-assessment questionnaire which were filled up by the participants and the measured amylase activity data were stored in the computer for further analysis.

Salivary amylase activity assay

Salivary α-amylase activity was estimated using the method outlined in the standard World Health Organization manual (George S et al 2018). In this procedure, 1.0 ml of buffered starch substrate was dispensed into three separate test tubes designated for the sample, control, and reagent blank. All tubes were pre-incubated in a water bath at 37 °C for 5 minutes to equilibrate the contents. Subsequently, 20 µl of the subject's saliva was introduced into the sample tube, thoroughly mixed, and incubated at 37 °C for precisely 7 minutes and 30 seconds. (No saliva was added to the control or blank tubes.) At the end of the incubation period, 1.0 ml of working iodine solution was immediately added to both the sample and reagent blank tubes, followed by 8 ml of distilled water. The mixtures were thoroughly homogenized, and absorbance readings were recorded without delay at 660 nm using a red filter (Ilford No. 608), with the spectrophotometer zeroed using distilled water as reference.

RESULTS

Sample characteristics

Thirty healthy students from first year dental college BDS participated in the study including both male & female (i.e. six and twenty-four) respectively. Table I shows the age and gender variations in our study. Table II shows the mean salivary amylase activity as well as mean score value of two genders of different classes of students. When stress score was analysed, as a function of gender, the values was found to be 106.3 and 95.7 for male and female respectively. Table III shows Average sAA activity and stress score in different groups of students as a function of gender.

Results shows that the stress score of students before the examination is above average level and reduced after the examination. Table IV shows the results of the statistical analysis, using paired-t test. The results indicated that there is a significant large difference between before the examination levels (M = 97.8, SD = 14.7) and After the examination levels of salivary amylase (M = 81.6, SD = 16.3), t (29) = 7.8. The values are statistically significant.

Table 1.

GENDER	SAMPLE 30	AGE	SAMPLE 30
Female	24	17 Year old	01
Male	6	18 Year old	11
		19 Year old	15
		20 Year old	03

Table 2.

	Mean SAA levels pre- exam	Mean SAA levels post- exam
Average	97.8	81.6
Male	106.3	87.5
Female	95.7	80.1

Table 3.

	Stress score	Mean SAA levels pre- exam	Stress score	Mean SAA levels post- exam
Average	75.96±5.8	97.8	65±1.0	81.6
Male	66.75±9.8	106.3	59.05±1.5	87.5
Female	80.05±7.1	95.7	71.16±1.3	80.1

Table 4.

STUDY GROUPS	Mean	S.D	Average of differences (x̄d)	Std. Error Mean	Lower	Upper	t	Two side p	p value
sAA levels pre internal exams	97.8	14.7	-16.27	0.3834	-16.6534	16.6534	29	**-7.761049**	< .001
sAA levels post internal exams	81.6	16.3							

DISCUSSION

The rising prevalence of stress across human populations has emerged as a global concern, affecting nearly every aspect of daily life. Despite significant research efforts, the identification of a highly sensitive, reliable, and non-invasive biochemical marker for objective stress assessment remains incomplete. The present study aimed to evaluate the influence of stress on salivary α-amylase (sAA) activity among first-year dental students at Rajas Dental College and Hospital, Tirunelveli District, Tamil Nadu, India, by comparing pre- and post-examination stress levels and validating sAA as an indicator of stress.

The findings revealed that students exhibited significantly higher stress scores prior to internal examinations, indicating heightened stress during this period. This observation aligns with previous studies demonstrating an increase in stress levels during academic assessments, accompanied by a corresponding elevation in salivary α-amylase activity (Chatterton et al., 1996). Recent investigations have further shown that sAA concentrations rise under stressful conditions and vary proportionally with stress intensity (Takai et al., 2004; Miyakawa et al., 2006; Nater et al., 2006; Lewis, 2006; Hebert and Lupien, 2007; Wagner et al., 2010; Hellhammer et al., 2009).

Analysis of the present data did not reveal any significant gender-related differences in stress scores, suggesting that the stress experienced by students in higher education is independent of sex. This elevated stress may be attributed to the transition from school-based to college-level examination patterns, which introduce new academic demands and evaluation methods. Correlation analysis demonstrated a strong positive association between stress scores and sAA activity ($p < 0.01$), and regression analysis confirmed that stress score was a significant predictor of sAA variation ($p < 0.01$). These findings corroborate earlier reports, underscoring the clinical relevance of salivary α-amylase as a robust, non-invasive biomarker for stress assessment. Furthermore, sAA activity represents a valuable tool for monitoring autonomic nervous system activity and quantitatively evaluating stress levels in academic and clinical settings.

CONCLUSION

This study demonstrates that salivary α-amylase activity reliably reflects acute stress levels among first-year dental students, particularly during examination periods. The strong positive correlation between stress scores and sAA activity highlights its potential as a sensitive, non-invasive biomarker for objective stress assessment and monitoring of autonomic nervous system function. These findings support the clinical and academic utility of sAA measurement and warrant further research to validate its applicability across diverse populations and educational settings.

REFERENCES

1. Ali N, Nater UM. Salivary Alpha-Amylase as a Biomarker of Stress in Behavioral Medicine. Int J Behav Med., Jun. 2020; 27(3): 337-342. d
2. Ali SQ, Hadi R. An Assessment of Alpha-Amylase as Salivary Psychological Stress Marker in Relation to Temporomandibular Disorders among a Sample of Dental Students. J Bagh Coll Dent, 2015 Dec.
3. BFEC (2011) Stress questionnaire. Retrieved November 10, 2011from http:// bfec.kenyon.edu /Healthy_Kenyon/ stress_psymptoms.pdf.
4. Chatterton R T J, Vogelsong K M, Lu Y C, Ellman A B and Hudgens G.A. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin. Physiol*, 1996; 16: 433–448.
5. George S, Joseph BB. Level of stress and its causes among 1st year dental students - A cross-sectional

study. Natl J Physiol Pharm Pharmacol, 2018; 8(11): 1518-1521.
6. Hebert S and Lupien S.J. The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.*, 2007; 411: 138–142.
7. Hellhammer D H, Wust S and Kudielka B M. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrino.* 2009; 34: 163–171.
8. Lewis J G. Steroid Analysis in Saliva: An overview. *Clin. Biochem. Rev.*, 2006; 27: 139–146.
9. McKay C A K, Buen E J, Bohan J K and Maye P J. Determining the Relationship of Acute Stress, Anxiety, and Salivary a- Amylase Level With Performance of Student Nurse Anesthetists During Human-Based Anesthesia Simulator Training. *AANA Journal*, 2010; 78: 4.
10. Miyakawa M, Matsui T, Kishikawa H, Murayama R, Uchiyama I, Itoh T, Yoshida T. Salivary chromogranin A as a measure of stress response to noise. *Noise Health*, 2006; 8: 108–113.
11. Nater U M and Rohleder N. Salivary alpha amylase as a noninvasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrino*, 2009; 34: 486-496.
12. Nater U M, La Marca R, Florin L, Moses A, Langhans W, Koller M M and Ehlert U. Stress-induced changes in human salivary alpha-amylase activity: associations with adrenergic activity. *Psychoneuroendocrinol*, 2006; 31: 49-58.
13. Nater U M, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C and Ehlert U. Human salivary alpha amylase reactivity in a psychosocial stress paradigm. *Int. J. Psychophysiol*, 2005; 55: 333 342.
14. Shkulaku R. Student's stress in higher education institutions: a critical review of foreign literatures and the ones in albania. *European Scientific J.* 2015; 1857–7881.
15. Steptoe A and Kivimäki M. Stress and cardiovascular disease. *Nature Rev. Cardiol*, 2012; 9: 360-70.
16. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K and Nishikawa Y. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Arch. Oral Biol.*, 2004; 49: 963-968.
17. Wagner J, Cik M, Marth E, Santner B I , Gallasch E, Lackner A and Raggam R.B. Feasibility of testing three salivary stress biomarkers in relation to naturalistic traffic noise exposure. *Int. J. Hyg. Environ. Health*, 2010; 213: 153-5.
18. Yamaguchi M, Kanemori T, Kanemaru M, Takai N, Mizuno Y and Yoshida H. Performance evaluation of salivary amylase activity monitor. Biosens. Bioelectron, 2004; 20: 491-497.