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Salivary Stress Biomarkers During Academic Examinations in Second-Year Dental Students

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ABSTRACT

Objective: Academic assessments are recognized as potent sources of stress for dental students. This study aimed to evaluate changes in salivary stress biomarkers—cortisol, α-amylase, and chromogranin A—associated with academic examinations in second-year dental students.

Methods and Materials: In this pre–post observational study, 40 second-year dental students (aged 20–22 years) from Al Hadi University College of Dentistry participated voluntarily. Unstimulated whole saliva was collected on two occasions: during a non-assessment period, 1 week before examinations, and during the assessment period at the end of exams. Samples were obtained by passive drooling for 5 minutes, coded to ensure confidentiality, and stored at −18 °C until analysis. Salivary cortisol, α-amylase, and chromogranin A concentrations were measured using ELISA according to the manufacturer's instructions. Paired t-tests were used to compare biomarker levels between the two time points, with $p \leq 0.05$ considered statistically significant.

Findings: Significant differences were observed in salivary cortisol and chromogranin A between the non-assessment and assessment periods, with higher mean levels in the non-assessment period compared with the examination period. In contrast, salivary α-amylase showed no statistically significant change between the two time points. These results indicate that cortisol and chromogranin A are sensitive to changes across the academic assessment period. In contrast, α-amylase did not reflect these changes in this sample of dental students.

Conclusion: Academic examinations in second-year dental students are associated with measurable alterations in salivary cortisol and chromogranin A, supporting their use as non-invasive biomarkers of academic stress. Monitoring these biomarkers may help educators identify high-stress periods and design interventions to reduce stress and promote students' psychological well-being.

Keywords: Salivary biomarkers, academic stress, cortisol, dental students.

Introduction

Stress is a state of worry or mental tension caused by a difficult or unbearable situation. It can also be defined as a natural human response that allows persons to overcome life challenges and difficulties (Rocha et al., 2013).

Academic stress affects students' psychological and physiological well-being. Stress requires an internal (psychological) balance in the body, along with the effort needed to adapt to it (Crnković et al., 2018). Dental students often suffer from increased anxiety, depression, and interpersonal sensitivity because of their studies, including theoretical and practical parts (Pani et al., 2011).

The stress has been shown to correlate with several salivary biomarkers. The choice of salivary biomarker to examine the body's response to academic stress depends on the type of stress studied. Chronic stress is associated with the activation of the hypothalamic–pituitary–adrenal (HPA) axis (measured by salivary cortisol), as well as with the depression of immune function (measured by salivary IgA and lysozyme). Acute stress is associated with activation of the sympatho–adrenomedullary system, which is reflected by salivary α-amylase and chromogranin A (Pani et al., 2011).

Saliva cortisol

Cortisol is thought to enter saliva in many ways, independent of an active transport mechanism or by passive diffusion (Kirschbaum & Hellhammer, 1994). It reflects the secretory activity in the hypothalamic-pituitary-adrenal (HPA) axis. Its level in saliva is less than that in blood. Salivary Cortisol correlates more closely with the free, physiologically active serum cortisol fraction than with total serum cortisol, which contains the physiologically inactive protein-bound cortisol fraction (Takatsuji et al., 2008). The main advantage of salivary cortisol over serum cortisol measurement is that the fear of the needle reduces stress, which may otherwise bias the results (Meeran et al., 1993). Salivary cortisol secretion, like serum cortisol, displays a marked diurnal rhythm, with its lowest level during slow-wave nocturnal sleep. An increase during late sleep, peaking after awakening. Levels then sloped rapidly, followed by a sustained, gradual decrease for the rest of the day (Edwards et al., 2001).

Salivary a-amylase is one of the main enzymes in humans and is released from the salivary glands in response to adrenergic stimulation. A-Amylase breaks down starch, first to oligosaccharides and then to maltose and glucose, by hydrolyzing a-1,4 glucan bonds. Amylase is also present in the pancreas, fallopian tubes, lung, prostate, and ovarian tissues (Koh & Koh, 2007).

It has an immunological function, protecting the oral cavity from microorganisms (Scannapieco et al., 1993). Salivary alpha-amylase has also been recognized as a marker that is sensitive to stimuli activating the sympathetic system (e.g., adrenaline) (Ali & Nater, 2020). In healthy individuals, alpha-amylase levels are lowest in the early morning and highest in the late afternoon (Takai et al., 2004). In response to stress, the concentration of alpha amylase in saliva rose rapidly, making it one of the essential salivary biomarkers of stress (O'Donnell et al. 2009), especially during blood sampling (Strahler et al., 2010). Acute stress activates the axis of the sympathetic nervous system, adrenal medulla, which is reflected not only in the concentration of salivary alpha-amylase, but also in the concentration of salivary chromogranin A (Chojnowska et al., 2021; Engeland et al., 2016).

Chromogranin A (CgA)

CgA is an acidic glycoprotein released from the adrenal medulla and sympathetic nerve endings that can be measured in the saliva (Nagasawa et al., 1998; NISHIKAWA et al., 1998).

Its secretion is increased from the submandibular salivary gland in response to different stress factors, e.g., public speaking (NAKANE et al., 1998), sports (Lee et al., 2006), noise (Miyakawa et al., 2006; Ng et al., 2003), passing an academic exam (Takatsuji et al., 2008), medical intervention (Obara & Iwama, 2005; Hua et al., 2014), or driving a car (Ng et al., 2003). However, there are no reports on the level of salivary chromogranin A in people diagnosed with depression or anxiety. Still, the main advantage of determining salivary CgA is that CgA levels are not affected by the time of day. Although its concentrations reach a peak shortly after waking, they drop rapidly (within only one hour) and remain constant throughout the day (NAKANE et al., 1998).

Methods and Materials

Study design and setting

This investigation was designed as a pre–post observational study carried out at Al Hadi University College of Dentistry. The study aimed to evaluate changes in salivary stress biomarkers associated with academic examinations in second-year dental students.

Participants and sampling

The study sample consisted of 40 second-year dental students aged 20-22 years enrolled in the Bachelor of Dental Surgery program at Al Hadi University College of Dentistry. Students were invited to participate voluntarily after receiving an explanation of the study objectives and procedures, and written informed consent was obtained from all participants. Only students who were available for both saliva collection sessions were included in the final analysis.

Saliva collection procedure

Saliva samples were collected on two occasions from each participant: the first was obtained 1 week before the examination period (non-assessment condition), and the second was collected at the end of the examination period (assessment condition). On each occasion, students were instructed to rinse their mouths with water to remove food debris and then wait for a short period before sampling. Unstimulated whole saliva was collected by allowing it to pool in the mouth and gently drooling it into a sterile, labeled collection tube. The tubes were immediately placed in an ice box and subsequently stored at −18 °C until analysis. According to the manufacturer's instructions, all samples were analyzed within 7 days of collection.

Biochemical analysis

Salivary concentrations of cortisol, α-amylase, and chromogranin A were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols. All assays were performed in duplicate to improve measurement reliability.

Statistical analysis

For each biomarker, values obtained before and during the examination period were compared using paired statistical tests (e.g., a paired t-test), with $p < 0.05$ as the level of significance. Data analysis was performed using standard statistical software.

Findings and Results

Table 1 shows the comparison of salivary chromogranin A levels before and after the academic assessment period. The mean chromogranin A level before the exams (M = 462.62, SE = 9.64) was higher than after the exams (M = 434.15, SE = 7.01). A paired t-test indicated that this difference between the two time points was statistically significant, t(39) = 23.684, p = 0.019, demonstrating a significant pre–post change in chromogranin A across the assessment period.

Table 1.

Comparison of salivary chromogranin A levels before and after academic assessment (n = 40)

Time point	Mean ± SE (ng/mL)	t-value	p-value
Before exams	462.62 ± 9.64		
After exams	434.15 ± 7.01	23.684	0.019

Note: Paired t-test; SE = standard error. S: Significant (p ≤ 0.05).

Table 2 presents the comparison of salivary amylase levels before and after the academic assessment period. The mean amylase level slightly decreased from before the exams (M = 14.24, SE = 0.55) to after the exams (M = 13.10, SE = 0.37). However, the paired t-test revealed that this difference was not statistically significant, t(39)= 1.384, p = 0.095. These findings indicate that salivary amylase did not differ significantly between the non-assessment and assessment conditions in this sample.

Table 2.

Comparison of salivary amylase levels before and after academic assessment (n = 40)

Time point	Mean ± SE (U/L)	t-value	p-value
Before exams	14.24 ± 0.55		
After exams	13.10 ± 0.37	1.384	0.095

Note: Paired t-test; NS: Non-significant (p > 0.05); SE = standard error.

Table 2 presents the comparison of salivary amylase levels before and after the academic assessment period. The mean amylase level slightly decreased from before the exams (M = 14.24, SE = 0.55) to after the exams (M = 13.10, SE = 0.37). However, the paired t-test revealed that this difference was not statistically significant, t(39) = 1.384, p = 0.095. These findings indicate that salivary amylase did not differ significantly between the non-assessment and assessment conditions in this sample.

Table 3. *Comparison of salivary cortisol levels before and after academic assessment (n = 40)*

Time point	Mean ± SE (μg/L)	t-value	p-value
Before exams	26.33 ± 0.56		
After exams	24.31 ± 0.71	1.785	0.027

Note: Paired t-test; S: Significant (p ≤ 0.05); SE = standard error.

Table 3 summarizes the comparison of salivary cortisol levels measured before and after the academic assessment period. The mean cortisol level before the exams (M = 26.33, SE = 0.56) was higher than after the exams (M = 24.31, SE = 0.71). Results of the paired t-test showed that this difference was statistically significant, t(39) = 1.785, p = 0.027, indicating a significant pre–post change in salivary cortisol associated with the examination period.

Discussion and Conclusion

Biological indicators for stress reactions are valuable markers in psychophysiological research. Academic examination stress is reported to increase physiological and psychological measures of stress and to decrease immune functioning. Psychosocial stress is widely known to induce various adaptive responses in physiologic systems, with particular increases in the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system. Cortisol levels reflect the HPA activity, whereas SAA and chromogranin A are said to reflect the SAM (Tammayan et al., 2021).

Estimation of salivary stress biomarkers was conducted during the assessment exam (stress period) and 1 week before the exam. This study clearly shows that there is a significant difference in the levels of cortisol and chromogranin A stress biomarkers between the assessment and non-assessment periods, implicating higher stress during exams. Salivary cortisol levels were notably less during the non-assessment period and increased significantly during the assessment period (p=0.000). Cortisol is a major glucocorticoid in humans that reflects adrenocortical activity. Activation of the HPA axis and subsequent cortisol release are significant components of the physiological stress response.

Salivary cortisol accurately reflects serum cortisol (Špiljak et al., 2024). Many studies have reported increased cortisol levels during stressful situations such as academic examinations, cardiac surgeries, and dental treatment procedures (Inder et al., 2012; Pruessner et al., 1997; Tammayan et al., 2021).

Alpha amylase

The salivary enzyme alpha-amylase has been proposed as a marker of stress-induced sympathetic nervous system activity.

It shows a nonsignificant difference between the two readings, which does not agree with Tammayan et al. (2021) and Ouda et al. (2016).

Application of noradrenaline or a beta-adrenergic agonist can stimulate sAA release, which acts as an indicator of sympathetic activity. A variety of studies on stress have consistently found increased levels of sAA in response to stress, and it is also generally used as a biomarker of stress (Tammayan et al., 2021; Ali & Nater, 2020).

Our study shows no significant difference due to its low durability in response to stress since the time between the onset of a mental stressor and the peak in its level is very quick, between 60 seconds and 180 seconds, and has a rapid recovery (Obayashi, 2013). It may be related to chronic stress, not acute one, according to Vineetha et al (Vineetha et al., 2014).

Chromogranin A

Chromogranin A is increased in secretion after the exam because stress is activated by the sympathetic nervous system, consistent with Takatsuji et al. (2008); Tammayan et al. (2021).

Its level is also increased during venipuncture in hospitalized children, which could be considered a good stress maker according to Lee et al.

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Declaration of Interest

The authors of this article declared no conflict of interest.

Ethical Considerations

The study protocol adhered to the principles outlined in the Helsinki Declaration, which provides guidelines for ethical research involving human participants. Ethical considerations in this study included the fact that participation was entirely optional.

Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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Authors' Contributions

All authors equally contribute to this study.

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