

Dynamic Changes in Salivary Cortisol and Protein among Dental Students

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Abstract

Background: Student assessments are the traditional methods of assessing academic success, and they are considered to affect one's career. This study evaluated the levels of salivary stress biomarkers represented by cortisol and total salivary protein during final academic assessments of dental undergraduates.

Methods: Saliva samples were obtained, one before the exam another afterwards. Concentrations of salivary stress biomarkers were obtained by enzyme-linked immunosorbent assay (ELISA).

Results: Before the exam, the two parameters were dramatically higher than afterwards, with a substantial difference between the levels of salivary protein and cortisol ($p=0.000$, 0.000 consecutively).

Conclusion: Stress induced by academic examinations may increase the level of salivary stress biomarkers in the short term.

Keywords: Academic stress; cortisol; ELISA; saliva; salivary stress biomarkers

Introduction

Academic exams are considered to be among the most challenging experiences for students, since passing or failing typically has implications for career development¹. In particular, two primary systems or locations in the brain are involved in the stress response: the sympatho-adrenomedullary and system hypothalamus-pituitary-adrenocortical axis¹. Increased secretion of cortisol in the adrenal cortex occurs due to the activation of HPA. Therefore, salivary cortisol represents the activity of HPA and is a more effective assessment than blood collection in stress research, which can induce spurious increases in cortisol secretion, representing the hyper-stress component¹. A wide range of data has revealed that several kinds of psychological

stress can result in HPA activation, leading to cortisol release and subsequently significantly higher salivary cortisol levels than resting baseline levels. The principal glucocorticoid in the human adrenal cortex is cortisol, which is synthesised from cholesterol. Higher levels of cortisol as a response to biochemical stress contribute to the well-characterised suppression of HPA related to health events and cognition¹. Salivary cortisol is present in a stable, unbound form and is the only fraction of hormones that display metabolic activity in combination with unbound plasma cortisol in the blood. Unbound cortisol reaches cells through passive diffusion due to lower of molecular weight and lipophilic nature, making it is possible to measure free cortisol in many body fluids. Up to 95% of secreted cortisol attaches to large protein molecules, like albumin and it is transported in the blood in the body. Salivary proteins have important functions, including the health of the oral cavity; the nutrition, survival and colonisation of microorganisms; and the adhesion and aggregation of microorganisms. Moreover, greater concentrations of total protein in response to stress lead to changes in saliva chemical properties, including oral surface adhesion or lubrication,

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and viscosity. Stress levels vary in different areas of education and learning, and higher stress levels could undermine the cognitive functions and learning abilities of students¹. Moreover, the prevalence of depression, anxiety and psychological distress among medical students is increasing¹. To investigate this phenomenon, this study used salivary cortisol as a biomarker for stress assessment during academic examinations, with higher levels found prior to a written test and its anticipation. Many studies have reported a rise in stress hormones (levels of cortisol) in anticipation of stressful experiences, such as oral exams, cardiac surgery and dental-treatment². Moreover, the response to physical or psychological stress in the body increases the cortisol secretion. Stress can be beneficial, of course, as it can boost drive and energy to get through stressful situations, such as examinations and work deadlines^{3,4}. Students face numerous academic problems in today's highly competitive world, however, including exam stress, lack of interest in a class and failure to understand a subject⁵. Exam stress is the feeling of fear or anxiety over one's performance in examinations⁶, and academic stress can increase students' anxiety levels⁷. Interest has been rising in identifying and using biomarkers in saliva as a more evaluative way to measure stress. The investigation of stress biomarkers has achieved recognition because saliva sample collection is standardised, non-invasive and easy to manage. Studies have shown that saliva can be used in chair-side tests for many oral and systemic diseases⁸. Saliva is useful because of its many analytes that are affected by a variety of conditions and physiological and pathological stressors⁹. Therefore, this study was designed to confirm salivary cortisol and total protein levels during psychological stress⁹ among undergraduate students in a dental college.

Materials and methods

From 2016–2017, a cross-sectional study has been conducted at the College of Dentistry, University of Anbar, Iraq. A total of 12 undergraduate students (6

males and 6 females) aged 21–23 years were randomly selected and recruited from different academic years to study. The study's objective and protocol were explained to all the recruited students, and their voluntary consent was obtained prior to participation. Two unstimulated saliva samples were collected from each student. In order to decrease the presence of food debris and consequent salivation stimulation, students were asked not to eat and drink water only about an hour prior to sample collection. The first sample was taken 30 minutes before a written examination at 8:30 am, and the second sample was taken at 12:00 pm after the examination was complete. Each student was instructed to rinse his or her mouth to remove debris. De-ionised water was used to rinse the mouth, and the participants were then asked to spit for 5 minutes in a special sampling container. The container was labelled with a collection number (1 or 2), date and time. All salivary samples were centrifuged at 3,000 rpm for 10 min to isolate pure saliva. Total protein and cortisol concentrations were calculated using a special kit (SPINREACT, Spain) with an enzyme-linked immunosorbent assay, as directed by the manufacturer.

Statistical Analyses

The data analysis was performed using version 11.0 of SPSS. A *p*-value smaller than 0.05 was determined as statistically significant. To check for discrepancies in the categorical variables, a Chi-square test was used.

Results

A total of 12 dental students (6 male, 6 female) from different academic years were recruited for this research to measure their levels of total free salivary cortisol (ng/ml) and total protein content in saliva. As shown in Figures 1 and 2, both parameters were significantly higher before the exam than afterwards, with a substantial difference between salivary protein and cortisol levels ($p=0.000$, 0.000 consecutively).

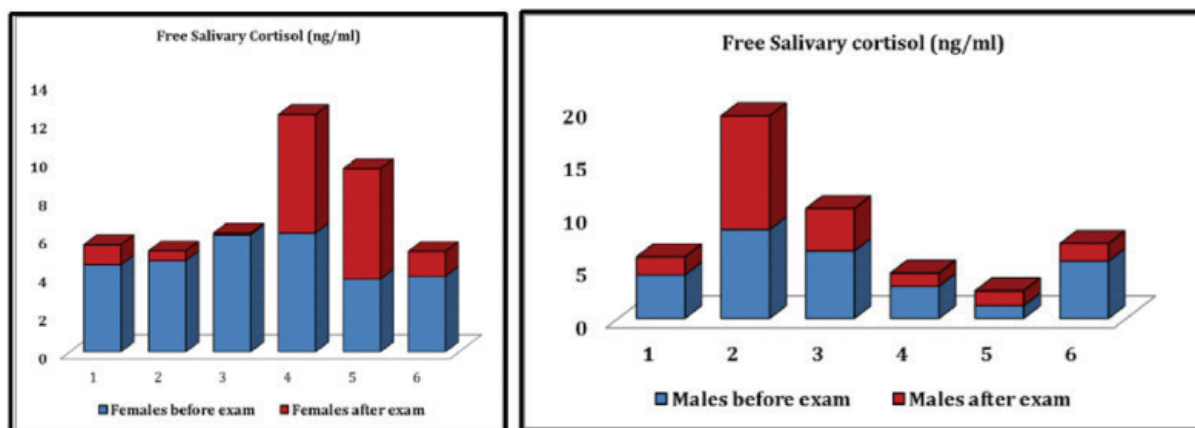


Figure 1: Levels of total free salivary cortisol (ng/ml) before and after the exam.

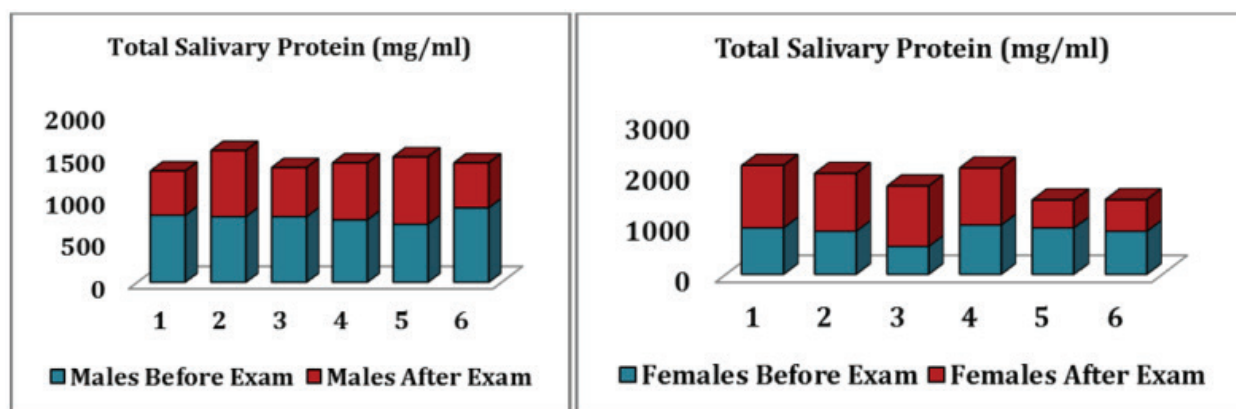


Figure 2: Levels of total free salivary protein (mg/ml) before and after the exam.

Discussion

Saliva plays an important role in the maintenance of oral hygiene. Various studies have shown that salivation changes often occur due to stress. In this study, we correlated the levels of certain parameters in saliva with the stress induced by an examination in medical students. Protein levels in saliva were significantly higher before the examination than afterwards, a finding consistent with that of Nauvoma et al., who also showed a significant increase in protein concentrations in saliva immediately after stress exposure¹⁰. Another study by Al-Nuaimy et al. showed similar findings by estimating protein concentrations before an oral academic exam and after one month of holiday break¹¹. To understand the mechanism behind this increased protein concentration, the activation of salivary glands as a sympathetic intervention during exposure to stress to control protein secretion has also been explored^{10, 12}. The autonomic nervous system primarily controls

protein secretion mainly by three salivary glands: parotid, sublingual and submandibular. The release of protein from the submandibular glands and parotid is elicited by stimulating the sympathetic system, and protein release from the sublingual gland is usually elicited by stimulating the parasympathetic system¹³.

We also measured free salivary cortisol levels during a stress condition before and after the academic exam. During chronic stress, alteration in cortisol levels is prominent. Cortisol is a stress hormone that is synthesised in the cortex of the adrenal gland¹⁴. As discussed earlier, the measurement of cortisol in saliva is superior to that in serum because a salivary assay enables the measurement of unbound cortisol.

We observed significantly higher cortisol levels in saliva before the academic examination in both males and females than afterwards, showing a direct correlation between saliva cortisol levels and stress.

Other studies have reported similar findings, indicating that training during medical courses causes higher incidences of psychological stress in students and that academic examinations are major stressors for students, likely because their performance affects their future careers^{2,15}. This increased stress leads to increased levels of cortisol^{10,11,16}. The increase in cortisol levels is attributed to HPA axis activity, which is more intense during stress reactions. Hypothalamus-level stressors activate the secretion of CRH and AVP, which stimulate the frontal lobe of the pituitary gland to release adrenocorticotrophic hormone (ACTH). ACTH stimulates the synthesis of cortisol and its secretion in the adrenal cells. Stress reactions are exhaustive and damaging to the body; indeed, cortisol limits and minimises the catabolic and immunosuppressive effects of stress reactions through a negative feedback mechanism^{17,18}.

This study therefore demonstrates that the stress of academic examination affected important components of saliva, including protein and cortisol, which decreased after the exam was over. This suggests that these levels are a short-term response to stress.

Conclusion

This study has shown increased protein and cortisol concentrations in saliva before the commencement of an academic exam, which decreased after the exam was over, suggesting that stress precipitates short-term changes in saliva composition. Higher levels of cortisol during an examination can reduce stress during the examination.

Conflict of Interest: All the authors declare no conflicts of interest.

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Ethical Approval: The study was conducted in compliance with the Helsinki Declaration and with the approval of the Regional Committee on Ethics, which is represented by the Medical Ethics Committee of the Ministry of Health in Iraq.

References

1. Ng V, Koh D, Mok BYY, Chia S-E, Lim L-P. Salivary biomarkers associated with academic assessment stress among dental undergraduates. *J Dent Educ.* 2003;67:1091–1094. doi:10.1002/j.0022-0337.2003.67.10.tb03701.x.
2. Lacey K, Zaharia M, Griffiths J, Ravindran A, Merali Z, Anisman H. A prospective study of neuroendocrine and immune alterations associated with the stress of an oral academic examination among graduate students. *Psychoneuroendocrinol.* 2000;25(4):339–56. doi:10.1016/S0306-4530(99)00059-1.
3. Ghatol SD. Academic stress among higher secondary school students: A review. *Int J Adv Res Educ Technol.* 2017;4(1):38–41.
4. Mehfooz Q, Haider S. Effect of stress on academic performance of undergraduate medical students. *J Community Med Health Educ.* 2017;7:6. doi:10.4172/2161-0711.1000566.
5. Nikitha S, Jose TT, Valsaraj BP. Effectiveness of academic stress management programme on academic stress and academic performance among higher secondary students in selected schools of Udupi District. *Nitte Univ J Heal Sci.* 2015;5(4):9–12.
6. Nikitha S, Jose TT, Valsaraj BP. A correlational study on academic stress and self - esteem among higher secondary students in selected schools of Udupi District. *Nitte Univ J Heal Sci.* 2014;4(1):106–08
7. Acharya PR, Chalise HN. Self-esteem and academic stress among nursing students. *Kathmandu Univ Med J.* 2015;52(4):298–02.
8. Prester L, Protrka N, Macan J, Katunarić M. Salivary sCD14 as a potential biomarker of dental caries activity in adults. *Arh Hig Rada Toksikol.* 2017;68(4):315–21. doi: 10.1515/aiht-2017-68-2974.
9. Koduru MR, Ramesh A, Adapa S, Shetty J. Salivary albumin as a biomarker for oral squamous cell carcinoma and chronic periodontitis. *Ann Med Health Sci Res.* 2017;7(5):337–40.
10. Naumova EA, Sandulescu T, Bochnig C, et al. Dynamic changes in saliva after acute mental stress. *Sci Rep.* 2015;4(1):4884. doi:10.1038/srep04884.
11. Al-Nuaimy K, Al-Hamdani I, Tawfik N. Effect of

- stress on the composition and flow rate of saliva. *Al-Rafidain Dent J.* 2012;12(1):66–70. doi:10.33899/rden.2012.42633.
12. Carpenter GH. The secretion, components, and properties of saliva. *Annu Rev Food Sci Technol.* 2013;4:267–76. doi:10.1146/annurev-food-030212-182700.
 13. Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. *Oral Dis.* 2002;8(1):3–11. doi:10.1034/j.1601-0825.2002.10815.x.
 14. Lee DY, Kim E, Choi MH. Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. *BMB Rep.* 2015;48(4):209–216. doi:10.5483/BMBRep.2015.48.4.275.
 15. Vaidya PM, Mulgaonkar KP. Prevalence of depression, anxiety and stress in undergraduate medical students and its correlation with their academic performance. *Indian J Occup Ther.* 2007;39:7–10.
 16. Singh R, Goyal M, Tiwari S, Ghildiyal A, Nattu SM, Das S. Effect of examination stress on mood, performance and cortisol levels in medical students. *Indian J Physiol Pharmacol.* 2012;56(1):48–55.
 17. Pacak K. Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. *Endocr Rev.* 2001;22(4):502–548. doi:10.1210/er.22.4.502.
 18. Bozovic D, Racic M, Ivkovic N. Salivary cortisol levels as a biological marker of stress reaction. *Med Arch.* 2013;67(5):374–77. doi:10.5455/medarh.2013.67.374-377.