Salivary Stress Biomarkers-Are They Predictors of Academic Assessment Exams Stress?

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Abstract

Purpose: The present study was conducted on undergraduate dental students to asses and compare the levels of salivary stress biomarkers including cortisol, immulnoglobulin-A and α-amylase enzyme during periods of academic assessments and non-assessments and to relate these biomarkers to students' academic performance. Methods: Saliva samples were collected from undergraduate dental students; one before taking a final assessment exam and another during non-assessment period. Salivary stress biomarkers concentrations were obtained using Enzyme-linked Immunosorbent Assay (ELISA). Results: The level of salivary stress biomarkers including s-cortisol, α-amylase, and immulnoglobulin-A significantly increased during periods of assessment exams as compared to non-assessment (p=0,000, 0.001, and 0.003 consecutively). The study found a significant correlation between salivary α-amylase and academic performance especially among male students (p=0.008) and those in their final academic year (p=0.040). Conclusion: We conclude that the stress of academic assessment can markedly increase the level of salivary stress biomarkers. Students who show less academic performance generally depict higher levels of salivary α-amylase, especially male students and those in their final academic year.

Key Words: Alpha-amylases, Cortisol, Salivary biomarkers, Academic stress

Introduction

Assessment exams of various natures are common methods to evaluate the performance of students and their mastery of a subject. Failing or passing assessments are considered to have a great impact on one's future and career. Therefore, it has been found that periods of academic assessment can be stressful experiences for students [1]. It was shown that students who were highly stressed after an exam had poorer performance III. Stress levels vary between students in different educational and learning fields. Increased levels of stress can negatively affect cognitive functions and learning abilities of students [2]. Depression, anxiety and psychological distress were found to be more prevalent among medical students [3].

Dental undergraduate programs are considered to be some of the longest and most demanding programs with lots of requirements to be met [2]. It is commonly associated with considerable stress symptoms expressed by students [3-5]. The undergraduate dental training in Saudi Arabia typically requires six years of training; three pre-clinical and three clinical years in addition to one year of internship. It has been reported that stress-endured by students during their final dental year differs from that during the first year [6].

Stress could be defined as changes in the homeostasis [7,8]. These changes are incorporated by increased consumption of oxygen and glucose in response to catecholamines released after the activation of the sympathetic-adrenal-medullary-system (SAM) [9-11]. If the stress prolonged, the hypothalamic-pituitary-adrenal axis (HPA axis) stimulates the release of cortisol [12]. Cortisol is a hormone produced by the adrenal glands in response to stressful conditions. Under normal conditions, it shows an increase in the early morning hours, reaches a peak before awakening and decreases in the evening (diurnal variation) [13].

Salivary cortisol is shown to be a useful biomarker to assess stress in stressful environments including academic examinations [14-16]. The existing literature shows conflicting results regarding the relation between stress and cortisol [17]. Salivary immunoglobulin A (s-IgA) is an antibody that plays a role in prevention of infectious diseases [18-24]. It has gained interest in psychoimmunological researches as it has been shown to be affected by variations in stress levels [25]. The relationship between stress and s-IgA is also unclear possibly due to differences in the testing methodologies [26,27].

Salivary alpha-amylase enzyme (SAA) is the most notable of salivary enzymes, which breaks starch into maltose [28]. Its secretion is mainly regulated by the autonomic nervous system [29,30]. Recent investigations showed a significant increase in SAA enzyme level in response to acute stress [9,11,31-33]. Stress, at any level, could cause sleeping disorders and burn out if left unattended [3,4]. While positive stress helps students reach peak performance [19] low academic achievement has been linked to higher stress levels. Students, who use different stress coping strategies such as problem solving with seniors and friends, have better academic performance and success [18]. Identifying low-performing students due to examination stress can be beneficial to raise stress awareness and educate them about effective stress coping strategies III.

This study was aimed at assessing and comparing the levels of salivary stress biomarkers including cortisol, IgA and SAA during periods of academic assessments and non-assessments.

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It was additionally aimed at relating these biomarkers to students' academic performance as measured by their GPA.

Materials and Methods

This cross-sectional study was conducted at the Faculty of Dentistry, King Abdulaziz University (KAUFD), Jeddah, Saudi Arabia and was study was approved by the research ethics committee at KAUFD (Protocol Number 052-015). A total of 90 undergraduate students were randomly selected and recruited to the study from various academic years. The study aims and protocol was explained to all recruited students and their consent was taken. Of these students, 45 were males and 45 females (*Table 1*).

Table 1. *Demographics of the participants.*

Variables	Frequency	Percent
Gender:		
Males	45	50
Females	45	50
Academic year:		
4th	43	47.8
males	29	
females	14	
5th	23	25.6
males	6	
females	17	
6th	24	26.7
males	10	
females	14	
GPA:	Mean	Median
semester	4.11	4.2
cumulative	4.12	4.18

Students with significant medical history or those on current medications were excluded. Two unstimulated saliva samples were collected from each student. The first one was taken during the period of students' final assessments 30-60 minutes before a written exam (sample 1). The second sample (sample 2) was taken three months after sample 1during a non-assessment period (summer vacation).

The investigators instructed each student to rinse his/her mouth to eliminate debris, set in an up-right position and spit in special sampling container for 5 minutes. Each container was then labeled with the sample number (1 or 2), date and time of collection. The investigator had the students name list and each student was given a specific identification number which was written on the container to maintain participants' confidentiality. One hundred and eighty samples were refrigerated at 4°C awaiting analysis within 14 days. Concentrations of s-cortisol (0-30 ng/ml), s-IgA (0-1200 µg/ml), and SAA (0-500 U/ml) were assessed by Enzyme-linked Immunosorbent Assay (ELISA) from Euroimmune Germany, according to manufacturer's instructions [34]. These semester and cumulative GPA scores of all recruited students was obtained from their academic records to be used during data analysis. Semester GPA refers to students GPA during a specific academic semester while their cumulative GPA marks their collective scores during all dental academic years.

Statistical Analyses

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software, version 17 (Chicago Inc.). A chi-square test was used to test differences in categorical variables and for comparisons of the proportions of students stratified according to different parameters (e.g. gender). T-tests were used to test the significance of differences between numerical values. Pearson correlation coefficient and multiple linear regression used to assess the correlation between the levels of salivary stress biomarkers with periods of academic assessments and non-assessments.

Results

This study recruited a total of 90 dental students (45 of each gender) of different academic years as can be seen in *Table 1*. The comparison of salivary stress biomarkers during assessment and non-assessment periods is depicted in *Table 2*. It can be seen that the levels of all biomarkers significantly increased during periods of academic Assessments.

Table2. *Values of salivary stress biomarkers during assessment and non-assessment periods*

Salivary stress biomarkers	Value during assess. period	Value during non-assess. period	Paired t-test	df	p value
s-cortisol	1.46	5.12	9.69	89	0
s-α-Amylase	115.11	195.87	3.377	89	0.001
s-IgA	337.1	774.31	3.108	89	0.003

Table 3 shows the correlation of the levels of stress biomarkers with students' GPAs. The results show that academic performance was negatively correlated with SAA levels (semester GPA (p=0.034) and cumulative GPA (p=0.024)) i.e. students with higher GPAs had lower levels of SAA during exam periods. Although a negative correlation was found between SAA and GPA during non-exam periods the correlation level was not statistically significant. The levels of s-cortisol and s-IgA were not found to have any significant correlation with either semester or cumulative GPA.

Table 4 shows a breakdown of these correlations by gender and academic year. The table shows that the level of SAA during the exam period in male students was negatively correlated with both semester and cumulative GPA (p=0.008 and p=0.031, respectively). A similar correlation in female students was not seen. The correlation of levels of s-cortisol and s-IgA with GPA was found to be inconsistent and non-significant.

Looking at the associations of salivary biomarkers and academic year showed that SAA level during exams was negatively correlated with semester GPA in 6th year students (p=0.04) and close to being significant in 4th year students (p=0.053). A similar negative correlation was also found between the level of SAA during exams and cumulative GPA in 6th year students (p=0.018).

Table 3. *Correlations of the levels of salivary stress biomarkers with students' GPA scores.*

Salivary stress biomarker	Correlation with semester GPA	Correlation with cumulative GPA
	(p value)	(p value)
s-cortisol:		
Exam:	-0.022	-0.084
	-0.838	-0.443
Rest:	0.31	0.028
	-0.779	-0.797
s-α-Amylase:		
Exam:	-0.231	-0.244
	-0.034	-0.024
Rest:	-0.037	-0.094
	-0.739	-0.393
s-IgA:		
Exam:	0.034	0.005
	-0.758	-0.963
Rest:	-0.006	-0.064
	-0.953	-0.558

A multiple regression model was used to look at the predictive effect of salivary biomarkers on semester and cumulative GPA (*Table 5*). The model showed that gender was the only significant predictor for GPA. The performance of male students can be correlated with the SAA levels only during exam periods.

Table 4. *Correlation of levels of salivary stress biomarkers with students' GPA scores, by gender and academic year.*

	Semester GPA						**Cumulative GPA**					
	s-cortisol Coefficient		**s-α-amylase Coefficient**		**s-IgA Coefficient**		**s-cortisol Coefficient**		**s-α-amylase Coefficient**		**s-IgA Coefficient**	
	(p-value)		**(p-value)**		**(p-value)**		**(p-value)**		**(p-value)**		**(p-value)**	
	Asses	**Non-assess**	**Asses**	**Non-assess**	**Asses**	**Non-assess**	**Asses**	**Non-assess**	**Asses**	**Non-assess**	**Asses**	**Non-assess**
Males	-0.042	-0.066	-0.413	0.079 (0.628)	0.240 (0.136)	-0.036 (0.827)	-0.061 (0.709)	-0.038 (0.817)	-0.341 (0.031)	0.064 (0.693)	0.210 (0.193)	-0.059 (0.717)
	-0.796	-0.686	-0.008									
Females	0.207	0.148	0.054 (0.725)	0.095 (0.534)	-0.185 (0.224)	0.182 (0.232)	0.126 (0.41)	0.128 (0.400)	-0.115 (0.451)	0.084 (0.585)	-0.215 (0.156)	0.117 (0.446)
	-0.172	-0.332										
Academic year												
4th	-0.13	0.104	-0.308 (0.053)	-0.091 (0.576)	-0.009 (0.957)	-0.145 (0.374)	-0.175 (0.280)	0.121 (0.458)	-0.236 (0.143)	-0.158 (0.329)	-0.100 (0.540)	-0.190 (0.239)
	-0.424	-0.522										
5th	-0.057	-0.31	0.176 (0.434)	0.209 (0.350)	0.248 (0.267)	-0.189 (0.400)	-0.194 (0.388)	-0.279 (0.209)	0.190 (0.398)	0.240 (0.282)	0.276 (0.214)	-0.284 (0.200)
	-0.802	-0.16										
6th	0.237	0.041	-0.432 (0.040)	-0.112 (0.611)	-0.043 (0.844)	0.338 (0.114)	0.237 (0.277)	0.056 (0.800)	-0.487 (0.018)	-0.120 (0.586)	-0.004 (0.984)	0.305 (0.157)
	-0.277	-0.841										
All students	-0.022	0.031	-0.231	-0.037 (0.739)	0.034 (0.758)	-0.006 (0.953)	-0.084 (0.443)	0.028 (0.797)	-0.244 (0.024)	-0.094 (0.393)	0.005 (0.963)	-0.064 (0.558)
(General correlation)	-0.838	-0.779	-0.034									

Discussion

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. Test taking can produce elevated stress and anxiety, with subsequent negative influences on test performance. This has been a focus of prior research. However, only a few studies have explored objective measurement of academic stress, test performance by using all the three salivary stress biomarkers particularly in Saudi Arabia [2].

Salivary cortisol, salivary IgA and salivary alpha-amylase were reported to react to physiological and psychological stressors, so we set out to investigate the changes in these stress biomarkers by employing a reliable laboratory stress protocol to investigate the effect of stress during exams on the levels of salivary stress biomarkers and their association with the academic performance of the students measured by GPA.

Table 5. Results of multiple regressions.

	Variable	B-coefficient	t-test	p-value
Dependent variable	Gender	0.49	4.684	0.000*
	Academic year	-0.112	-0.96	0.34
	log s-cortisol exam	-0.02	-0.19	0.85
	log s-cortisol rest	0.111	1.038	0.303
	logs-α-Amylase exam	-0.1999	-1.806	0.075
	logs-α-Amylase rest	0.1222	1.119	0.267
	log s-IgA exam	0.091	0.874	0.385
	log s-IgA rest	0.046	0.434	0.267
Dependent variable	Gender	0.625	6.659	0.000*
	Academic year	-0.093	-0.89	0.376
	log s-cortisol exam	-0.052	-0.547	0.586
	log s-cortisol rest	0.066	0.684	0.496
	logs-α-Amylase exam	-0.175	-1.764	0.082
	logs-α-Amylase rest	0.054	0.55	0.584
	log s-IgA exam	0.061	0.65	0.518
	log s-IgA rest	-0.01	-0.107	0.915

Psychosocial stress is widely known to induce various adaptational responses of physiologic systems with particular increasing activities in the hypothalamus-pituitary-adrenal axis (HPA) as well as in the sympathetic-adrenal-medullary (SAM) system. Cortisol levels reflect the HPA activity whereas SAA ands-IgA are said to reflect the SAM activity [35]. Hence, all three biomarkers were taken into consideration in the present study as they are the established biomarkers of stress reflecting both the SAM and HPA activity [36-38].

In this within subject repeated measures design, estimation of salivary stress biomarkers was done during periods of assessment exams (stress period) and during summer vacation (rest period)This study clearly shows that there is a significant difference in the level of all stress biomarkers during assessment and non-assessment periods, clearly implicating the higher level of stress during exams.

Salivary cortisol levels were notably less during 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 and subsequent release of cortisol are major components of the physiological stress response. Salivary cortisol accurately reflects serum cortisol, the physiologically active component [39]. Some studies have reported increased cortisol levels during stressful situations such as academic examinations, cardiac surgeries and dental treatment procedures [14,15,40]. The concentration of s-IgA in saliva is considered to be a good indicator for stress and functional status of the mucosal immune system. Studies have shown that there is significant increase in the concentration of s-IgA in saliva during academic exams [41] and in response to acute stressors such as oral presentations, [42] public speaking, [43] and active mental stress tasks [44]. Similarly in the present study a significant rise in s-IgA was observed. Therefore, s-IgA can be used as s sensitive indicator of stress among individuals.

The salivary enzyme alpha-amylase has been proposed as a marker for stress-induced activity of the sympathetic nervous system. In the present study a significant rise in SAA was noted during academic assessment as compared to non-assessment periods. The increase of amylase concentrations is consistent with earlier studies on amylase stress responses [27,31,32]. Our results show that SAA, especially during assessment periods was the only biomarker significantly correlated with students' academic performance as measured by their GPA scores. This finding is vital as students' performance during academic assessment scan depend on multiple factors such as coping with stress. Animal and human studies have indicated that stress and glucocorticoids may impair memory and cognitive function [45-47]. These salivary biomarkers have the potential of being assessment tools to identify those students who are consistently stressed during academic assessments and who may perform poorly because of inability to cope with stress.

Stress has long been the focus of studying, but we are only beginning to understand its complexity. Through new techniques, researchers are now able to measure the response of the primary stress systems in a convenient, non-invasive manner, thus allowing research in this area to progress more rapidly. Stress research is essential as findings can be used to improve our quality of life. By gaining a thorough understanding of the systems governing stress response, we can develop intervention techniques designed to target over-activation of these systems, decrease stress, and improve health. The recent findings concerning s-cortisol, s-IgA and SAA can be a significant contribution to the much needed future research in the non-invasive and accurate objective measurement methods of assessing stress.

Conclusion

We conclude that assessment-related stress can markedly increase the level of salivary stress biomarkers. Dental students who show less academic performance generally depict higher levels of salivary α-amylase, especially male students and those in their final academic year.

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Conflicts of Interest

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