

Effect of Academic Exam Stress on Salivary Biomarkers in Dental Students

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Abstract: Academic examinations are a common source of psychological stress that may influence salivary secretion and biochemical markers. This study evaluated the effect of academic stress on salivary flow rate, pH, cortisol, α -amylase, and immunoglobulin A (IgA) levels in dental students from the College of Dentistry, University of Baghdad. Unstimulated saliva was collected from students before and after academic examinations. Salivary flow rate and pH were measured immediately, while cortisol, α -amylase, and IgA levels were analyzed using dry chemistry analysis. Data was statistically compared between pre- and post-examination samples. No significant differences were found in salivary flow rate, cortisol, α -amylase, or IgA levels before and after the exam, whereas salivary pH was significantly higher following the examination. Academic stress produced minimal physiological changes in the evaluated salivary parameters except for increased pH, which may reflect transient alterations in oral buffering activity. Further studies with larger samples are recommended to confirm these findings.

1 INTRODUCTION

Exploring salivary biomarkers has emerged as a promising avenue in the quest to improve the diagnosis of mental disorder and to monitor it. Saliva, an accessible and non-invasive biological fluid, presents a unique glance into the body's physiological aspect, refers to sum physiological and biochemical variations associated with mental health cases [1], [2]. These biomarkers include enzymes, hormones, antibodies, and other molecular structures that match with pathological variations in the brain and body accompanied these cases [3], [4]. Stress is characterized as a group of physiological and psychological actions that activate the mechanism of person's defense in reaction to a range of threats. In reaction to stress, the hypothalamus-pituitary-adrenal (HPA) axis is mainly stimulated [5]. When a person suffers from a stressful state, the psychological response to this state stimulates the sympathetic both the nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axis, leading to the secretion of rising amount of stress hormones, especially cortisol

and catecholamine, which results decrease in salivary flow [6]. Academic exams are the strongest stressors that students suffer from. Strong stress has been noticed to increase the activity of the hypothalamus-pituitary adrenal (HPA) axis with resulting rise in cortisol level [7]. Cortisol has been widely used to gauge the activity of the HPA stress axis and has received considerable attention in research related to stress physiology. Life situations are described as unpredictable or dangerous causing increased cortisol release. Cortisol secretion, which physiologically peaks upon waking and gradually declines throughout the day, increases in situations of acute stress before returning to normal levels after the stressor ceases. Studies indicate that acute stress can reduce immune system performance and inhibit immunoglobulin production. Salivary immunoglobulin A (sIgA) measurement is another potential non-invasive way for measuring stress. In saliva, IgA is the most common Ig isotype. In oral mucosal immunity, salivary IgA is the most important defensive antibody [8]. Salivary alpha-amylase has been determined as a possible biomarker for the autonomic

nervous system's response to stress, particularly sympathetic activity. Several studies have reported increased levels of salivary Alfa Amylase (AA) in response to physical stress (heat, cold and daily exercise) [9]. The present study aimed to investigate the association between the levels of salivary cortisol, Alpha-Amylase and sIgA and stress in second year students at College of Dentistry / University of Baghdad who have done Academic Exams and determine the correlation between variables.

2 EXPERIMENTAL

2.1 Study Design

Cross sectional study (before and after study).

2.2 Subjects

Forty subjects were enrolled in this study (40 subjects before the exam and the same subjects after the exam). Unstimulated saliva collection was carried out before and immediately after the exam according to the guidelines described by Tenovuo and Lagerlof [10]. Samples were centrifuged for 10 minute at 3000 rpm. The clear supernatant was collected in an Eppendorf tube and then stored at (-20° C) in a deep freeze until analysis.

2.3 Calculation of Salivary Flow Rate

All unstimulated saliva was collected from patients for 5 min. Volume was calculated and expressed as ml/min [11].

2.4 Determination of Saliva Ph

A digital pH meter with a single electrode was used to measure saliva pH (Jenway pH meter 3320) [12].

2.5 Detection of Salivary Cortisol, Amylase and Iga Levels

The concentrations of cortisol, amylase and sIgA were determined using the dry chemistry analysis method. Measurements were performed with an automated dry-chemistry analyzer (Getein 1180) following the manufacturer's instructions. This technique utilizes dry slide technology, in which multilayered slides contain dry reagents specific to each analyte. When the saliva sample is applied to the

slide surface, chemical reactions occur within the reagent layers, giving a color change proportional to the concentration of analyte. The color intensity color is measured photometrically, and the concentration is automatically calculated by the analyzer [13].

Each sample was analyzed in duplicate to ensure precision. Quality control materials were included in each analytical run to confirm accuracy and reliability. Reference intervals and analytical performance characteristics were in accordance with the manufacturer's specifications.

3 RESULTS AND DISCUSSIONS

The descriptive statistics for salivary parameters in both groups are presented in Table 1. The mean salivary pH in group 1 (before the exam) was 6.92 ± 0.23 , ranging from 6.0 to 7.0, while in group 2 (after the exam) it was 6.80 ± 0.31 , with the same range. The salivary flow rate showed a mean value of 0.87 ± 0.64 mL/min in group 1 and 0.85 ± 0.33 mL/min in group 2. Salivary cortisol levels demonstrated considerable variability, with a mean of 120.34 ± 60.63 µg/dL in group 1 and 149.28 ± 77.08 µg/dL in group 2. The mean salivary IgA concentration was 9.88 ± 2.36 mg/dL for group 1 and 9.38 ± 1.70 mg/dL for group 2. Amylase activity showed a mean value of 6.19 ± 7.17 U/mL in group 1 and 6.04 ± 7.33 U/mL in group 2.

A paired samples t-test was conducted to compare salivary parameters between the two study groups. The results demonstrated a statistically significant difference in salivary pH values between group 1 and group 2 ($p = 0.009$), indicating that the mean salivary pH was significantly higher in group 1.

No statistically significant differences were observed in salivary flow rate, cortisol concentration, IgA levels, or amylase activity ($p > 0.05$) as revealed in Table 2.

The relationships among salivary pH, flow rate, cortisol, IgA, and amylase levels among the students before exam were examined by Pearson's correlation analysis (Table 3). The results indicated that none of the correlations between the measured variables reached statistical significance ($p > 0.05$) in this group. Salivary pH showed a weak positive correlation with cortisol levels ($r = .33$, $p = .079$), and a very weak positive correlation with amylase ($r = .09$, $p = .662$), while it was negatively correlated with flow rate ($r = -.11$, $p = .566$) and IgA ($r = -.03$, $p = .897$). Flow rate was negatively correlated with cortisol ($r = -.06$, $p = .752$), IgA ($r = -.05$, $p = .811$), and amylase ($r = -.18$, $p = .346$). Cortisol demonstrated a

small negative association with IgA ($r = -.16$, $p = .405$) and amylase ($r = -.02$, $p = .919$). Similarly, IgA was very weakly correlated with amylase ($r = .04$, $p = .849$). Overall, these findings suggest that there were no statistically significant associations among salivary pH, flow rate, cortisol, IgA, or amylase levels in the students before exam ($N = 29$).

Regarding the correlation among the measured variables in students after the exam, the analysis showed that salivary amylase exhibited a significant positive correlation with sample number ($r = 0.527$, $p = 0.003$), indicating that amylase levels increased with later samples. Additionally, salivary IgA showed a significant negative correlation with amylase

($r = -0.377$, $p = 0.044$), suggesting that higher IgA levels were associated with lower amylase concentrations.

No other significant correlations were found among the variables studied. Specifically, salivary pH did not significantly correlate with flow rate ($r = 0.008$, $p = 0.967$), cortisol ($r = -0.117$, $p = 0.546$), IgA ($r = -0.038$, $p = 0.844$), or amylase ($r = 0.144$, $p = 0.457$). Likewise, salivary flow rate showed no significant associations with cortisol ($r = -0.212$, $p = 0.270$), IgA ($r = 0.129$, $p = 0.503$), or amylase ($r = 0.054$, $p = 0.782$). Cortisol levels were also not significantly correlated with any other parameter as shown in Table 4.

Table 1: Descriptive statistics for study groups.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
pH1	29	6	7	6.92	0.227
pH2	29	6	7	6.80	0.308
Flow Rate 1	29	0.31	3.00	0.8725	0.63548
Flow Rate 2	29	0.37	1.70	0.8476	0.33306
Cortisol1	29	41	337	120.34	60.627
Cortisol2	29	39	422	149.28	77.082
IgA1	29	6	14	9.88	2.355
IgA2	29	6	13	9.38	1.699
Amylase1	29	1	32	6.19	7.173
Amylase 2	29	1	30	6.04	7.334

Table 2: Paired samples test.

Paired Samples Test						
	Paired Differences			t	df	Sig. (2-tailed)
	Mean± Std. Deviation	95% Confidence Interval of the Difference				
		Lower	Upper			
pH1 - pH2	0.121± 0.232	0.032	0.209	2.801	28	0.009
FlowRate1 - FlowRate2	0.02490 ± 0.70443	-.24305	0.29285	0.190	28	0.850
Cortisol1 - Cortisol2	28.931 ± 78.810	-58.909	1.047	1.977	28	0.058
IgA1 - IgA2	0.493± 2.906	-0.612	1.599	0.914	28	0.369
Amylase 1 – Amylase 2	0.154± 2.562	-0.820	1.128	0.324	28	0.748

Table 3: The correlations among the measured variables in students before exam.

Correlations						
		pH1	Flow Rate1	Cortisol1	IgA1	Amylase1
pH1	Pearson Correlation	1	-0.111	0.332	-0.025	0.085
	Sig. (2-tailed)		0.566	0.079	0.897	0.662
Flow Rate 1	Pearson Correlation	-0.111	1	-0.061	-0.046	-0.182
	Sig. (2-tailed)	0.566		0.752	0.811	0.346
Cortisol 1	Pearson Correlation	0.332	-0.061	1	-0.161	-0.020
	Sig. (2-tailed)	0.079	0.752		0.405	0.919
IgA1	Pearson Correlation	-0.025	-0.046	-0.161	1	-0.037
	Sig. (2-tailed)	0.897	0.811	0.405		0.849
Amylase 1	Pearson Correlation	0.085	-0.182	-0.020	-0.037	1
	Sig. (2-tailed)	0.662	0.346	0.919	0.849	

Table 4: The correlations among the measured variables in students after exam.

		Correlations				
		pH2	Flow Rate 2	Cortisol 2	IgA2	Amylase 2
pH2	Pearson Correlation	1	-0.008	-0.117	-0.038	0.144
	Sig. (2-tailed)		0.967	0.546	0.844	0.457
Flow Rate 2	Pearson Correlation	-0.008	1	-0.212	0.129	0.054
	Sig. (2-tailed)	0.967		0.270	0.503	0.782
Cortisol 2	Pearson Correlation	-0.117	-0.212	1	0.066	-0.083
	Sig. (2-tailed)	0.546	0.270		0.735	0.669
IgA2	Pearson Correlation	-0.038	0.129	0.066	1	-0.377*
	Sig. (2-tailed)	0.844	0.503	0.735		0.044
Amylase 2	Pearson Correlation	0.144	0.054	-0.083	-0.377*	1
	Sig. (2-tailed)	0.457	0.782	0.669	0.044	

The protective functions of saliva and the relationship between psychological effects and oral disease are studied. Surprisingly, a little psychological stress can affect defense systems in saliva. The current study found that no statistically significant alterations were noticed in salivary flow rate, salivary cortisol concentration, sIgA levels, or salivary amylase activity between two groups (before and after exam). This finding may suggest that the level or duration of academic stress was insufficient to elicit measurable changes in salivary gland activity. Additionally, the high inter-individual variability and possible adaptation of students to recurrent examination stress could have contributed to the absence of significant differences. Moreover, salivary secretion is influenced by several confounding factors such as hydration status, time of sampling, and dietary habits, which might have masked subtle stress-induced alterations. This result disagrees with the study done by Al-Nuaimy and her team [14] since they found a decrease in salivary flow rate. They attributed the cause to the sympathetic stimulation gives little saliva including high protein concentration and may thus give a sensation of dryness. Subjective oral dryness and reduced unstimulated salivary flow were significantly accompanied with state anxiety, perceived stress, depression, and trait anxiety [15]. On other hand, this study comes in line with the research done by Takatsuji and his colleagues; they also found no change in the level of cortisol before and after examinations and contrary with investigation reported increased cortisol secretion in response to academic examination [16]. At the same time Takatsuji's results disagree with the results of current study since they found the concentration of IgA increase immediately after the exam [17]. Moreover, the results of the present study disagree with results of study conducted by Abed since he found an elevation of amylase level with a significant change.

He attributed the cause to the fact that some persons are more affected by some stress factor than others; so, the person's characteristics and behavior styles must be considered to determine their worth and their vulnerability to stress [18]. Regarding the pH results, this study is consistent with the study done by Al-awadei and Qasim, they found that salivary pH was not significantly lower in their studied groups [19]. No significant correlations were found among salivary pH, flow rate, cortisol, α -amylase, and IgA levels before and after the examination period. This may indicate that these parameters are regulated by different physiological mechanisms and do not necessarily respond synchronously to psychological stress. Moreover, individual variability, timing of sample collection, and uncontrolled external factors such as hydration and circadian rhythm could have contributed to the absence of significant associations.

4 CONCLUSIONS

The present study investigated the effects of academic examination stress on salivary biomarkers, including cortisol, α -amylase, and immunoglobulin A (sIgA), as well as salivary flow rate and pH, among second-year dental students at the University of Baghdad. The findings revealed no statistically significant differences in salivary flow rate, cortisol, α -amylase, or sIgA concentrations before and after examination, except for a slight but significant reduction in salivary pH following the exam. This minor pH decline may reflect transient changes in oral physiology under mild stress conditions rather than a pronounced stress response. The absence of significant alterations in major biomarkers suggests that the level or duration of academic stress experienced by participants was insufficient to trigger measurable activation of the hypothalamic-pituitary-adrenal (HPA) axis or the

autonomic nervous system. Additionally, inter-individual variability, adaptation to repetitive exam-related stress, and the influence of external factors-such as hydration, diet, and circadian rhythm- may have minimized detectable physiological responses. Overall, the study indicates that short-term academic stress may not markedly influence salivary biochemical markers in healthy young adults. However, longitudinal investigations involving larger, sample sizes, multiple stress levels, and extended observation periods are recommended to better understand the complex relationship between psychological stress and salivary biomarkers.

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