Effect of Stress on the Composition and Flow Rate of Saliva

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الخلاصة

الأهداف: لتحديد تأثير الشد النفسي الحاد على وظائف الغدد اللعابية. **المواد وطرائق العمل:** شارك في هذه الدراسة 23 طالبا من كلية طب الأسنان حيث تم جمع عينتين من اللعاب الغير محفز لمدة 5 دقائق. العينة الأولى تم أخذها قبل أداء احد الامتحانات الشفهية لنصف السنة، واخذ النموذج الثاني بعد شهر في وقت ليس فيه أي شد نفسي(كمجموعة سيطرة). تم حساب سرعة جريان اللعاب في الدقيقة الواحدة عند اخذ العينتين وكذلك تم قياس تركيز البروتين، والألبومين، وحمض البول، والكالسيوم في اللعاب باستخدام الطرائق التشخيصية الخاصة بكل فحص المأخوذة من شركة بايولابو (فرنسا). **النتائج:** أظهرت النتائج وجود انخفاض معنوي لتركيز الكالسيوم(0,23 ± 0,21ملي مول/لتر) نتيجة الشد النفسي بنسبة – 78,50%. كما أدى الشد النفسي إلى انخفاض تركيز الألبومين (13,39±16,39 ملي غرام/100مل) بنسبة (- 37,30%) مع قلة في نسبة جريان اللعاب في الدقيقة الواحدة (– 4,62%)، وزيادة تركيز البروتين(1,03± 0,69 غرام/100مل) وحمض البول(1,67 ±1,13 ملي مول/لتر) بنسبة (9,57%، 10,60% على التوالي) علما بأن جميع هذه التغيرات كانت غير معنوية عند مقارنتها مع العينة الأخرى (مجموعة السيطرة). **الاستنتاجات:** أظهرت الدراسة بأن الشد النفسي الحاد يؤثر على تركيز مكونات اللعاب ونسبة جريانه وهذا مما يؤكد على دور اللعاب كسائل عضوي مهم في الحفاظ على صحة الفم والأسنان.

ABSTRACT

Aims: To assess the effect of acute psychological stress on some salivary glands functions. **Materials and Methods:** Twenty-three undergraduate dental students participated in this study. They were asked to provide two samples of unstimulated whole saliva for 5 minutes, the first sample before amid- year oral academic examination and subsequently the second, one month later in a non stressful situation (after holiday break) as control group. Salivary flow in one minute was determined, in addition, total protein, albumin, uric acid and calcium levels in saliva were assessed using determination kits method from Biolabo (France). **Results:** The results showed a significant stress mediated decrease in the salivary calcium concentration (0.23±0.21 mmol/L) by -78.50% in comparison with non stressful condition. Salivary flow level (0.62 ± 0.28 ml /min) and albumin concentration (16.39±13.69 mg/100ml) were decreased by (- 4.62% and -37.30% respectively), while uric acid (1.67±1.13 mmol/L) and total protein concentrations (1.03±0.69 g/100ml) were elevated by 9.56% and 10.60% respectively. Not all these changes were statically significant. **Conclusions:** These results suggest that the acute psychological stress exerts its influence on salivary composition and this will increase the value of saliva as dynamic biological fluid in controlling the oral health.
Key Words: Saliva, stress, total protein, albumin, uric acid, calcium.

INTRODUCTION

Academic examination have been considered as one of the most acute stresses experienced by students because passing or failing usually has consequences for one's career development.[1-3] Changes in salivation often accompany the stress response, therefore it is important to estab-lish whether these changes is truly mirror the physiological response to stress, or merely confound altered salivary flow.[3,4] Salivary gland secretion is mainly under autonomic nervous control, but various hormones may also modulate salivary composition.[5] Saliva consists of two components that are secreted by inde-

pendent mechanisms; first component includes ions, which is produced mainly by parasympathetic stimulation and second protein component, which is released mainly in response to the sympathetic stimulation.[6] Saliva plays an important role in oral health monitoring, regulating and maintaining the integrity of the oral hard tissues and some soft tissues; many studies have demonstrated the importance of salivary calcium with regard to both dental and gingival health.[7] The association between stress and the occurrence of oral disease is based on clinical observations, epidemiological research, and experiments with animals.[8-10] Saliva possesses a multiplicity of immunological and nonimmunological defense systems against toxins, fungi, viruses, and bacteria.[7, 8] The importance of saliva to the oral health becomes evident in individuals with a reduced salivary flow, particularly in the dry mouth syndrome or xerostomia.[6,11] One of the symptoms of xerostomia is a dramatic increase of dental caries and other oral infections, accompanied by an increase of pathogenic bacteria.[12]Human saliva lubricates the oral cavity structures and protects teeth and oral mucosa against potentially injurious factors.[6, 7] Protection of the oral tissues is, among other things, achieved by the physical movement of saliva that effectively washes away many potentially harmful microorganisms.[6,8,10] This study of dental undergraduate students will assess the effect of examination stress on salivary flow and on concentration of some salivary constituents and to assess the differences between these parameters before the examination, and after the holiday.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Faculty of Basic Science of Dentistry, College of Dentistry, University of Mosul. During a mid year term test in physiology. Twenty three undergraduate dental students of either sex participated in this study, age range from 21-23 years with a mean age of 21 ± 1.12years. None of the participants was under medication and all reported to be in good health, most of the students were non-smoker (74%).

Students were requested in advance not to eat or drink except for water one hour before saliva collection to minimize possible food debris and stimulation of salivation. The data were collected on two occasions; the first occasion was obtained on the first day of the exam week and the second sample was collected after 6 weeks of the first measurement, during a period of rest (after mid-trimester holiday) as a control. Five minutes unstimulated whole saliva was collected.[13] Deionized water was used to rinse the mouth. The volume of the saliva was measured and divided by five minutes to obtain the salivary flow rate expressed in ml/min. After the collection, the samples were centrifuged (4000 rpm, for 10 minutes). The supernatant was divided over several aliquots and frozen at (-20°C) until analysis. Whole saliva was assessed colorimetrically by a spectrophotometer. Calcium, uric acid, total protein and albumin concentration were determined by (O-Cresolphtalein complexone, Uricase, Biuret and Bromocresol methods respectively) using a commercial kits (Biolabo reagents, Biolabo SA France).[14]

Data analysis was performed using SPSS version 13.0 for Window. Data were expressed as the mean ± standard deviation (M±SD), and percentage (%) throughout the paper. Means were compared using an paired sample Student's t-test. The relation between the salivary flow and biochemical parameters was explored by means of Pearson correlation coefficient(r). $P<0.05$ was considered a statistically significant difference.

RESULTS

In this study, the saliva of 23 dental students was assessed on two occasions. During examination stress, the results showed non significant increase of uric acid and total protein concentrations (1.67 ± 1.13 and1.03 ± 0.69) by (10.60% and 9.57% respectively) compared with the non stressful sample. While salivary flow rate (0.62 ± 0.28) and albumin levels (16.39 ± 13.69) were decreased by (- 4.62%, - 37.30% respectively), all these results were statistically non significant (Table 1).

Table (1): Comparison between the effect of exam stress and non-stress (holiday samples) situation on salivary flow rate and biochemical parameters.

Parameter	Exam Stress Samples Mean ± SD N=23	Non Stress Samples Mean ± SD N=23	% of changes	*P* value
Salivary flow rate (ml /min)	0.62 ± 0.28	0.65±0.30	-4.62%	.216
Ca (mmol/L)	0.23± 0.21	1.07±0.99**	-78.50%	.001
Total protein (g/100ml)	1.03±0.69	0.94±0.59	9.57%	.644
Uric acid (mmol/L)	1.67±1.13	1.51±1.23	10.60%	.546
Albumin (mg/100ml)	16.39±13.69	21.92±10.76	-37.30%	.216

Significant at ** $p < 0.01$

The concentration of calcium was significantly decreased (-78.50%) at level of $p<0.01$ in comparison with the non-stressful condition (control group) as shown in figure (1):
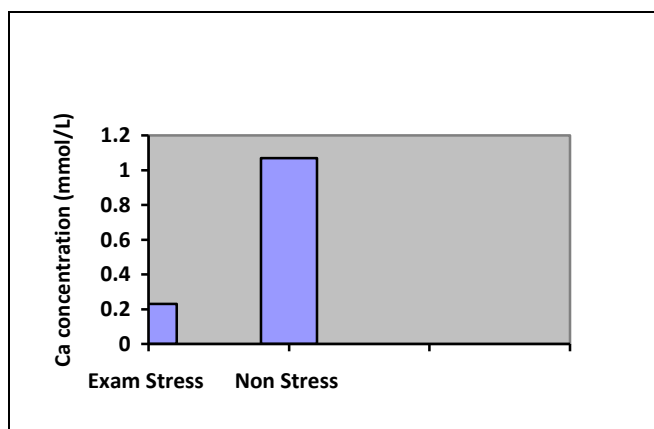

Figure (1): Comparison between mean Calcium concentration in exam stress sample and Non-stress sample

Correlation analysis between these parameters in the first sample (stress induced) showed that there were interrelation between the parameters but it's not statistically significant (Table 2), while correlation analysis between the same parameters in the second sample (holiday) (Table 3) showed that salivary flow were negatively correlated with uric acid concentration (r = - 0.46, $p< 0.05$) (Figure 2).

Table (2): Correlations between salivary flow rate and biochemical parameters in exam stress situation

Parameter	Salivary flow rate (ml /min)	Ca (mMol/L)	Total protein (g/100ml)	Uric acid (mMol/L)	Albumin (mg/100ml)
Salivary flow rate (ml/min)	-				
Ca (mmol/L)	-.187	-			
Total protein (g/100ml)	-.261	.124	-		
Uric acid (mmol/L)	-.240	-.321	-.040		
Albumin (mg/100ml)	-.059	.186	-.340	-.091	-

Table (3): Correlations between salivary flow rate and biochemical parameters in Non Stress situation

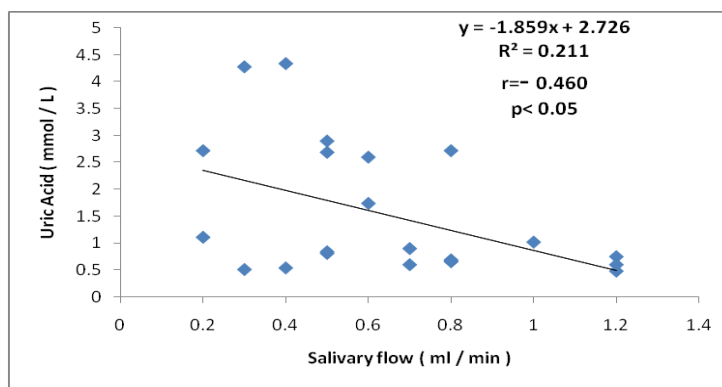| Parameter	Salivary flow rate (ml /min)	Ca (mMol/L)	Total protein (g/100ml)	Uric acid (mMol/L)	Albumin (mg/100ml)
Salivary flow rate(ml/min)	-				
Ca (mmol/L)	-.052	-			
Total protein (g/100ml)	-.198	.037	-		
Uric acid (mmol/L)	-.460*	-.108	.321		
Albumin (mg/100ml)	-.070	.094	.261	.137	-

Figure (2): Correlation between salivary flow rate and uric acid concentration in Non-stress Sample

DISCUSSION

The protective functions of saliva and the association between oral disease and psychological factors is considered. It is surprising how little psychological stress can alter the defense systems in saliva. In this study, total protein concentration was elevated, while salivary flow rate decreased and this is in agreement with other studies.[2-4] Turner and Sugiya (2002) suggested that Parasympathetic stimulation produces copious saliva of low protein concentration while sympathetic stimulation produces little saliva but of high protein concentration and may thus give a sensation of dryness.[5,15] Subjective oral dryness and reduced unstimulated salivary flow were significantly associated with depression, trait anxiety, perceived stress and state anxiety.[10,11] This elevation could be caused by an increased sympathetic tone and catecholamine output,[4] or could be due to activation of the hypothalamic-pituitary- adrenal axis and subsequent release of cortisol in saliva with subsequent increase in total protein content, and secretory immunoglobulin A as one defense mechanism(2). Calcium is one

of important inorganic content of salivary fluid.[16] Secretion of calcium in saliva depend upon salivary flow rate, so there is a negative correlation between calcium concentration and salivary flow rate[17] and this is in agreements with the results of the study. Sewon *et al*. (1998) showed that a positive correlation between high salivary calcium content and periodontitis and between high salivary calcium level and the number of intact teeth in selected groups of subjects.[17] Change in the concentration of some of the saliva constituent is co-responsible for the dynamics of the processes, which contribute to the development of new equilibrium between teeth remineralization and demineralization.[7] Uric acid is considered as one of the constituents in the salivary fluid. The concentration of uric in mixed saliva has been reported as a range from 0.5 to 20.6 mg/100ml[18] and this is in agreement with our results. In recent years it has been emphasized that the concentration of many of the constituents of saliva varies with flow rate and that composition of the saliva produced by individual salivary gland differs from each other's (19, 20). Uric acid is

one of the antioxidant defenses and that the elevation in the levels could be due to this cause, uric acid content in saliva correlate with plasma uric acid.[20]

CONCLUSION

This study demonstrates that the effect of stress (mid-year examination) on some important constituent of saliva. The results suggest that acute psychological stress exerts its effect on salivary composition and this will increase the value of saliva as dynamic biological fluid in controlling the oral health.

REFERENCES

1. Kiecolt-Glaser JK, Garner W, Speicher C, Penn GM, Holliday J, Glaser R. Psychosocial Modifiers of immunocompetence in medical students. Pychosomso Med 1984; 46:7-14.
2. Ng V, Koh D, Betty YY, Chia SE, Lim L. Salivary biomarkers associated with academic assessment stress among dental undergraduates. *J Dent Educ*. 2003; 67: 1091-1094.
3. Chiappelli F, Iribarren FJ, Prolo P. Salivary biomarkers in psychobiological medicine. *Bioinformation*. 2006; 1: 331- 334.
4. Bosch JA, Brand HS, Ligtenberg TM, Bermond B, Hoogstraten J, Amerongen AVN. Psychological stress as a determinant of protein levels and salivary-induced aggregation of streptococcus gordonii in human whole saliva. *Pychosomso Med*. 1996; 58: 374-382.
5. Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. *Oral Dis*. 2002; 8: 3–11.
6. Scully CBE. Salivary glands and saliva, drug effects on salivary glands: dry mouth. Oral Diseases. 2003; 9: 165–176.
7. Surdacka A, Strzykata K, Rydzewska A. Changeability of oral cavity environment. *Eur J Dent*. 2007; 1: 14-17.
8. Dean VS, Simon CL. Periodontal disease is associated with lower Anti oxidant capacity in whole saliva and evidence of increased protein oxidation. *Clinl Scie*. 2003, 105:167-172.
9. Bayazit V. Evaluation of Cortisol and Stress in Captive Animals. *Austr J Basic Appl Scie* 2009; 3(2): 1022-1031.
10. Lawrence HP. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J Can Dent Assoc*. 2002; 68(3):170-174.
11. Bergdahl M, Bergdahl J. Low unstimulated salivary flow and subjective oral dryness: association with medication, anxiety, depression, and stress. *J Dent Res*. 2000; 79: 1652–1658.
12. Edgar WM, Higham SM, Manning RH. Saliva stimulation and caries. Prevention. *Adv Dent Res*. 1994; 8(2): 239-245.
13. Navazesh M. Method for collecting saliva. *Ann Ny Acad Sci*. 1993; 694: 72-77.
14. Burtis CA, Ash wood ER. Tietz text book of clinical chemistry 3rd ed. WB Saunders Co. Philadelphia. 1999; Pp: 477- 530, 1245-1250, and 1395-1406.
15. Carlson GW. The salivary glands. Embryology, anatomy, and surgical applications. *Surg Clin North Am*. 2000; 80: 261–273.
16. Carey CM, Vogel GL. Measurement of calcium Activity in oral fluids by Ion selective Electrode: Method evaluation and simplified calculation of Ion Activity products. *J Res. Natl. Inst. Stand Technol*. 2000; 105(2):267-273.
17. Sewon LD, Karjalainen SM, Soderling E, Lapinleimu H, Simell O. Association between salivary calcium and oral health. *J clin periodontal*. 1998; 25 (11): 915-9.
18. Shannon IL, Terry JM, Chauncey HA. Uric and total protein in serum and parotid fluid in relation to periodontal status. *J dent Res*. 1966; 25: 1539-1540.
19. Mason DK, Boyle JA, Harden RM, Duncan AM, Greig WR. The influence of flow rate on the concentration of uric acid in human parotid and submandibular saliva. *J dent Res*. 1966; 45: 1439-1444.
20. Barbara K, Smolenski RT, kanp N. Determination of adenine nucleotides and their metabolites in human saliva. *Acta Biochimica polonica*. 2000; 47 (3): 877-879.