Original paper

Biochemical Changing in Saliva Components during Tension Situations and Its Relationship with Age

Rana Fadhil Mousa^*

^College of pharmacy, Karbala University, Karbala, Iraq.

Abstract

Background: Several pathologies of the oral cavity have been associated with tension. The association between stress and the occurrence of oral disease is based on clinical observations, epidemiological research, and experiments with animals.
Aim of study: The aim of this study was to identify the effect of acute tension on some salivary glands functions, and relationship the age with this tension
Materials and Methods: Twenty three of students from AL-karama school of girls (age 7-12 year) and thirty undergraduate pharmacy females students (age 20-24 year) were participated in this study. They were asked to provide two samples of unstimulated whole saliva for 5 minutes, the first sample collected after a week end in the usual academic day as a control group, the second sample was collected after one month later in a first day of final examinations of a scholastic year**.** Total protein, albumin, uric acid and calcium levels in tow samples were assessed.
Results & Discussion: The results showed a significant stress mediated decrease in the salivary calcium concentration (2.8813 mg/dl) in comparison with non-stressful condition (6.8020 mg/dl). and albumin concentration (1.608 g/dl were decreased by comparison with normal value (2.818 mg/ dl), while uric acid (6.874 mg/dl in sample under stress, 4.330 mg/dl in normal sample) and total protein concentrations (75.20 g/l for girl without stress, 107.15 g/l under stress) were elevated respectively all these result in age range (20–24year). While in age range (7 – 12 year) the result was as a follow:- for uric acid the value for sample under stress was highly than in normal condition (5.118 mg/dl while to sample without tension is 3.200 mg/dl). level of albumin is (2.195 g/dl to girl under stress and 4.388 g/dl in normal condition) that men there is a significant decrease in albumin level .also the result showed a significant decrease change in calcium level (2.6707mg/dl while in normal sample is 5.038 mg/dl) also we showed a significant elevation level in total protein (81.567g/l) comparison with normal sample (68.600 g/l) all these changes were statistically significant as we showed. Our search also compared between the age group and its relationship with acute tension. So, the level of uric acid in age (7-12 and 20 -24) are (5.118 mg/dl , 6.874 mg/dl) sequential ,while non-significant change in level of albumin in both age group in opposite to value of calcium and total protein we showed a highly level in age between (20- 24 year) in comparison with (7-12 year).
Conclusion 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. Also this search suggest the effect of age to the composition of saliva.
Key words: Saliva, tension, Calcium, albumin, uric acid, total protein

Introduction

Several pathologies of the oral cavity have been associated with tension. These include conditions such as periodontal disease, acute necrotizing ulcerative gingivitis, dental caries, recurrent pathos ulcerations, and upper respiratory infections. The association between stress and the occurrence of oral disease is based on

*For correspondence E-mail ranafadhil78@yahoo.com

clinical observations, epidemiological research, and experiments with animals [(1)]. Academic examination have been considered as one of the most acute stresses experienced by students or candidate, 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 establish whether these changes is truly mirror the physiological response to tension, 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 independent 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.[(4,6)]

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 or tension and the occurrence of oral disease is based on clinical observations, epidemiological research, and experiments with animals.[(1,10)] Saliva possesses a multiplicity of immunological and non-immunological 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.[(11)] Human saliva lubricates the oral cavity structures and protects teeth and oral mucosa against potentially injurious factors.[(12)] Protection of the oral tissues is, among other things, achieved by the physical movement of saliva that effectively washes away many potentially harmful microorganisms.[(5,9,10)]

This study of pharmacy undergraduate students will assess the effect of examination tension on concentration of some salivary constituents and to assess the differences between these parameters before the examination, and after the week end 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 final exam and the second sample was collected after a period of rest (holiday of Friday, Saturday) as a control. Deionizer water was used to rinse the mouth. The volume of the saliva was measured after the collection, the samples were centrifuged (4000 rpm, for 10 minutes). The supernatant was divided over several aliquots and frozen until analysis Whole saliva was assessed by a spectrophotometer. Calcium, uric acid, total protein and albumin concentration were determined by (Biuret and Bromocresol methods respectively) using a commercial kits (human, Biolabo SA France).[(13)]

The aim of this study is to identifications the effect of acute tension in some salivary glands functions, and relationship the age with this tension

Materials and Methods

This study was conducted at AL-KARAMA primary school of girls in kerbala city, age range from (7-12 years) and undergraduate pharmacy girls students (age 20-24 years) During a second semesters participated in this study, none of the participants was under medication and all reported to be in good health, Students were requested in advance not to eat or drink except for water one hour be-fore 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 final exam period and the second sample was collected after week end, during a period of rest as a control. Deionized water was used to rinse the mouth. 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 (Biuret and Bromocresol methods respectively) using a commercial kits (human, and Biolabo SA France).

Statistical analysis

Data analysis was performed using SPSS (statistical Package for Social Sciences) version 13.0 for Window. Data were expressed as the mean±standard deviation (M±SD), Means were compared using a paired sample Student's t-test. $P<0.05$ was considered a statistically significant difference. [14]

Results

The results showed a significant stress mediated decrease in the salivary calcium concentration (2.8813mg/dl) in comparison with non-stressful condition (6.8020 mg/dl) and albumin concentration (1.608 g/dl were decreased by comparison with normal value (2.818 mg/ dl) while uric acid (6.874 mg/dl in sample under stress, 4.330 mg/dl in normal sample) and total protein concentrations (75.20 g/l for girl without stress,107.15 g/l under stress) were elevated respectively all these result in age range (20–24year) table (1). While in age range (7–12 year) the result was as a follow:- for uric acid the value for sample under stress was highly than in normal condition (5.118 mg/dl while to sample without tension is 3.200 mg/dl). level of albumin is (2.195 g/dl to girl under stress and 4.388 g/dl in normal condition) that men there is a significant decrease in albumin level .also the result showed a significant increase change in calcium level (2.6707 mg/dl while in normal sample is 5.038 mg/dl) also we showed a significant elevation level in total protein (81.567g/l) comparison with normal sample (68.600 g/l) table (2). All these changes were statically significant as we showed our search also compared between the age group and its relationship with acute tension. So, the level of uric acid in age (7-12 and 20-24) are (5.118 mg/dl , 6.874 mg/dl) sequential ,while non-significant change in level of albumin in both age group in opposite to value of calcium and total protein we showed a highly level in age between (20- 24 year) in comparison with (7-12 year) table 3 .

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.[2,8] 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.[6-9] 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 secretary immunoglobulin A as one defense mechanism.[2].

Table 1. Levels of parameters in normal and under stress samples in age group (7-12 year)

Parameters	sample	Mean	Std. Deviation	T value	P value
uric acid mg/dl	normal	3.2000	.85475	3.371	0.005*
	under stress	5.1187	1.63366		
Albumin g/dl	normal	4.3880	1.50096	3.08	0.027*
	under stress	2.1959	.92293		
Calcium mg/dl	normal	5.038	1.06021	6.33	0.001 **
	under stress	2.2533	0.41713		
total protein g/L	normal	68.600	8.59069	5.97	0.001**
	under stress	81.567	1.29847		

Table 2. Levels of parameters in normal and under stress samples in age group (20-24 year)

Parameters	sample	Mean	Std. Deviation	T value	P value
uric acid mg/dl	normal	4.3300	1.38894	3.05	0.011*
	under stress	6.8747	2.15230		
Albumin g/dl	normal	2.8180	.93959	2.65	0.016*
	under stress	1.6081	.86883		
Calcium mg/dl	normal	6.8020	.496761	1.85	0.081
	under stress	2.8813	2.04861		
total protein g/L	normal	75.2000	7.91833	4.47	0.007*
	under stress	107.1553	24.03590		

Table 3. Levels of parameters in under stress samples in both age group (20-24 year) and (7-12 year).

Parameters	Age	Mean ±SD	P value
uric acid mg/dl	7-12	5.1187	*0.02
	20-24	6.8747	
Albumin g/dl	7-12	2.1959	0.08
	20-24	1.6081	
Calcium mg/dl	7-12	2.6533	0.44
	20-24	2.8302	
total protein g/L	7-12	81.5673	*0.001*
	20-24	107.1553	

Statistical analysis by spss program
*Mean significant value ($p \leq 0.05$)
** mean high significant value ($p \leq 0.001$)

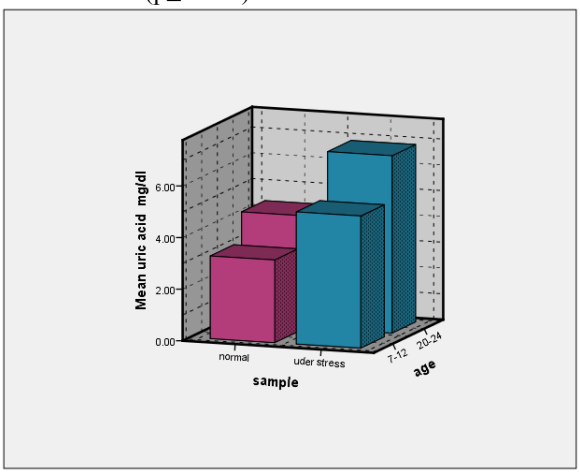

Figure 1. level of uric acid in normal and samples under stress for both age group, uric acid in sample under stress more than normal samples and in age (7-12) less than age (20-24)

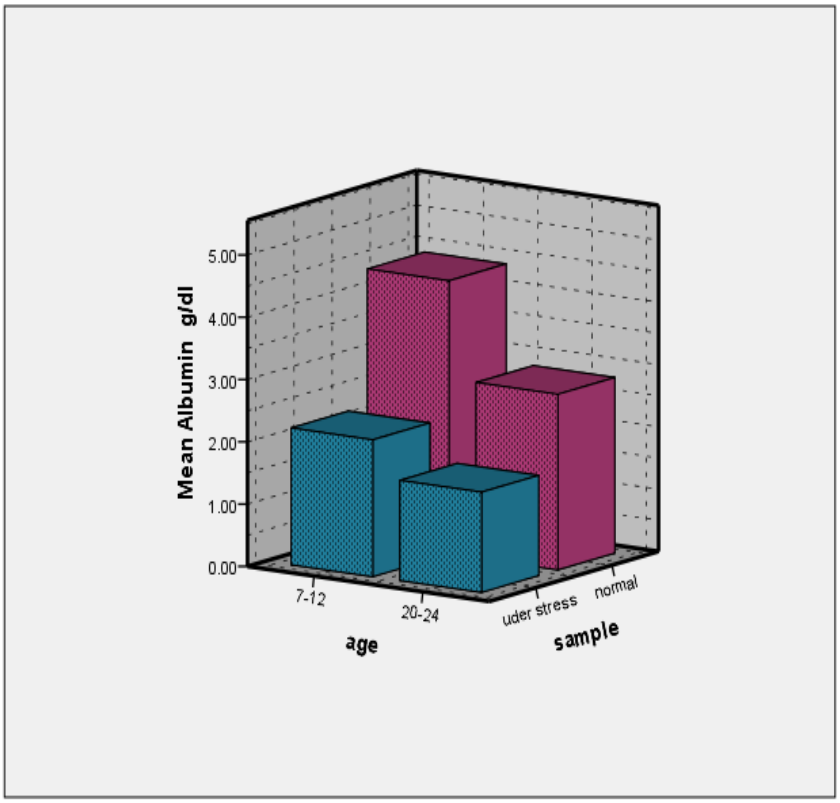

Figure 2. level of albumin in normal and samples under stress for both age group , albumin in sample under stress less than normal samples and in age (7-12) more than age (20-24)

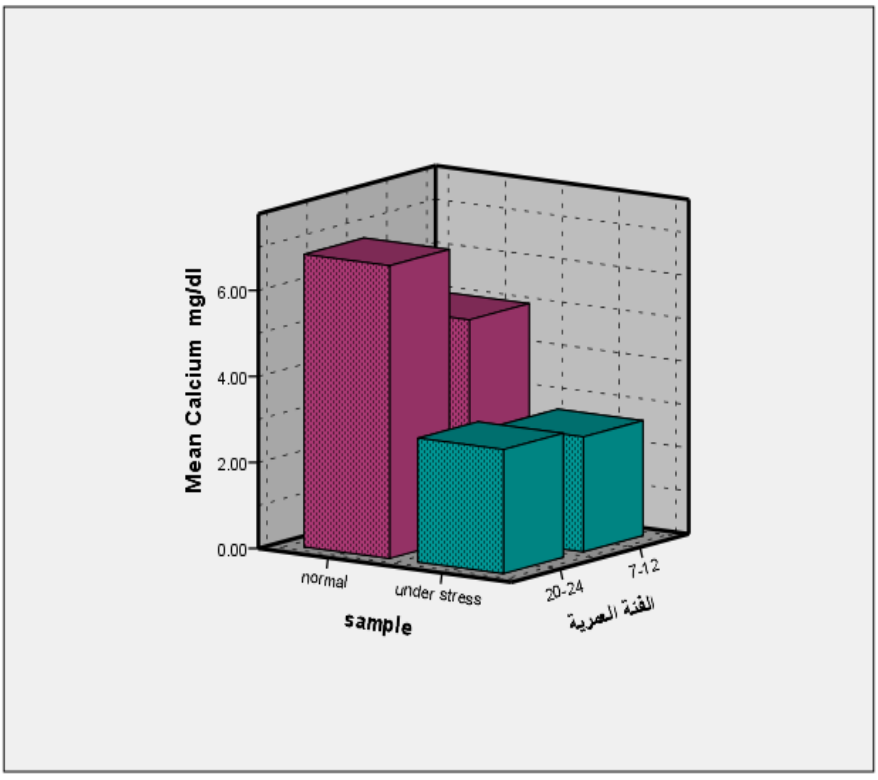

Figure 3. level of calcium in normal and samples under stress for both age group , calcium in sample under stress less than normal samples and in age (7-12) less than age (20-24)

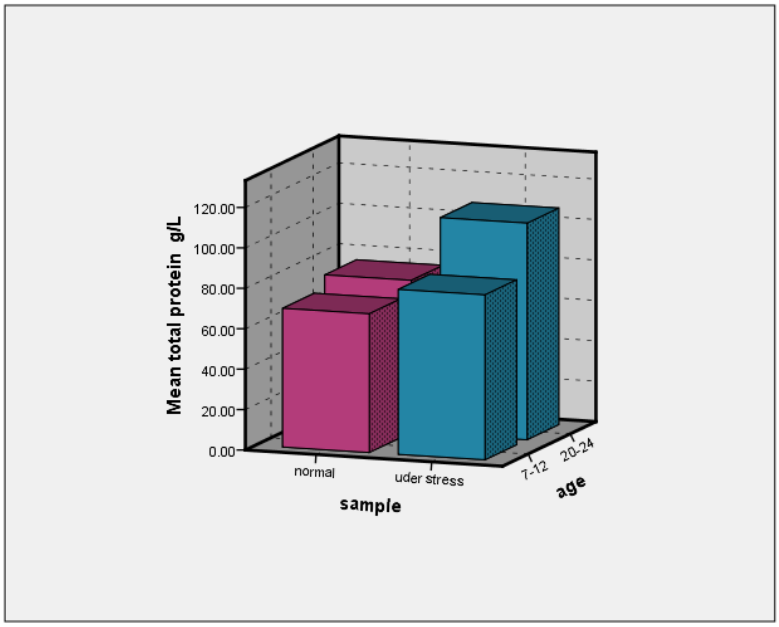

Figure 4. level of total protein in normal and samples under stress for both age group, total protein in sample under stress more than normal samples and in age (7-12) less than age (20-24)

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.[17,18] 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 [19]. 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.[21] albumin fined in sublingual gland and in the situation like stress ,depression, the rate of it's secretion was decrease so for this reason the level of albumin become less than normal value. [7]

Conclusion

This study demonstrates that the effect of stress (final 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. Also this search suggest the effect of age to the composition of saliva.

Reference

1. Schenkels LC, Veerman EC, Nieuw Amerongen AV. Biochemical composition of human saliva in relation to other mucosal fluids. *Crit Rev Oral Biol Med*. 1999,6:161–175.
2. Henskens YM, van der Velden U, Veerman EC, Nieuw Amerongen AV. Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis or

periodontitis. *J Periodontal Res*.1998, 28:43–48.
3. Meurman JH, Rantonen PJF. Salivary flow rate, buffering capacity, and yeast counts in 187 consecutive adult patients from Kuopio, Finland. *Scand J Dent Res*., 1992, 102:229–234.
4. Meurman JH, Collin H-L, Niskanen L, Töyry J, Alakuijala P, Keinänen S, et al. Saliva in non-insulin-dependent diabetic patients and control subjects: the role of autonomic nervous system. *Oral Surg Oral Med Oral Pathol*.2000; 86:69–76.
5. Samaranayake LP. Nutritional factors and oral candidosis. *J Oral Pathol*.1968, 15:61–65.
6. Turner and Sugiya .World Health Organization. Oral health surveys: basic methods. 3rd ed. Geneva: 2002, WHO.
7. World Health Organization. Manual to the international statistical classification of diseases, injuries and causes of death.1977, 9th rev Geneva: WHO.
8. Lussi A and Jaeggi Chemical factors; in Dental erosion from diagnosis to therapy (ed) A Lussi (Basel: Karger) 2006, pp 77–87.
9. Turner and Sugiya Erosion—diagnosis and risk factors. Clin. Oral. Inv.2008, 12 5–13.
10. Mantle M and Allen A, colorimetric assay for glycoproteins based on the periodic acid/Schiff stain. Biochem. Soc. Trans.2001 607–609.
11. Chang J-Y and Knecht R, Direct analysis of the disulfide content of proteins: Methods for monitoring the stability and refolding process of cystine-containing proteins. Anal. Biochem. 1997, 52–58.
12. Dawes C Stimulus effects on protein and electrolyte concentrations in parotid saliva. J. Physiol.1984, 346 579–588.
13. Attin T, Meyer K, Hellwig E, Buchalla W and Lennon AM Effect of mineral supplements to uric acid on enamel erosion. Arch. Oral. Biol2003, 48 753–759.
14. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD and Bairoch A Identification and Analysis Tools on the ExPASy Server; in The proteomics protocols handbook (ed) JMT Walker (New Jersey: Humana Press) 2005, pp 571–607.
15. Harmsen BJM, De Bruin SH, Janssen LHM, Rodrigues de Miranda JF and Van Os GAJ pK change of imidazole groups in bovine serum albumin due to the conformational change at neutral pH. Biochemistry2011, 10; 3217–3221.
16. Sutton PRN: Stress and dental caries. In Stable PH (ed), Advances in Oral Biology 2. New York, Academic Press, 1966, 104-148.
17. Sewon *et al*.:- Stress relaxation and saliva: Relationship to dental caries and its prevention. Ann Dent1998, 42:47-54.
18. Carey CM, Vogel GL. Measurement of calcium Activity in oral fluids by Ion selective Electrode: Method evalu-ation and simplified calculation of Ion Activity products. *J Res. Natl. Inst. Stand Technol*; 2000; 105:267-273.
19. Sewon LD, Karjalainen SM, Soderling E, Lapinleimu H, Simell O. Associa-tion between salivary calcium and oral health. *J clin periodontal*. 1998, 25: 915-9.
20. Shannon IL, Terry JM, Chauncey HA. Uric and total protein in serum and pa-rotid fluid in relation to periodontal status. *J dent Res*. 2010; 25: 1539-1540.
21. Turner RJ, Sugiya H. Understanding salivary fluid and protein secretion. *Oral Dis*. 2002; 8: 3–11.