Determination of the effect of stress on the salivary cortisol level among sample of university students having myofacial pain

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ABSTRACT

Background: Psychological stress is considered the major etiological factor precipitating myofacial pain and temporomandibular disorders.It is known that stress induce various adaptational responses of physiologic systems. The process includes increase in the activity of the hypothalamic-pituitary-adrenal axis which promotes cortisol secretion. Salivary cortisol has been used as a measure of free circulating cortisol levels.The use of salivary biomarkers has gained increased popularity since collecting samples is non-invasive and painless. The aim of thisstudy was to evaluate the level of cortisol in saliva among sample of university students having myofacial pain, during the final exam period and whether this finding could have a significant value as a symptomatic psychobiological marker.

Materials and Methods: Ninety eight (98) university students were enrolled in this study. Fifty(50) were with myofacial pain (symptomatic) and forty eight (48) were without myofacial pain (asymptomatic) as a control group.Each student with myofacial pain was examined according to Research Diagnostic Criteria for Temporomandibular Disorders. Saliva sample were collected from each subject before final examination and three months later for biochemical analysis of cortisolusing ELISA test.

Results: A highly significant difference in salivary cortisol level between the two periods for both the study and control groups, and a non-significant difference between the two groups in before examination period. A negative association has been observed between the level of salivary cortisol and severity of pain and a highly significant improvement of pain between the final examination periods and three months later.

Conclusions: Dental students perceived a higher level of stress prior to the final exam was associated with raised salivary cortisol levels which could be considered as a useful non-invasive biomarker for measuring acute stress.

Keywords: Stress, Cortisol, Myofacial pain. (J Bagh Coll Dentistry 2013; 25(3):87-90).

INTRODUCTION

Psychological factors, such as stress, were considered to play a major roles in the etiology, progression, and complications of temporomandibular disorders (TMDs).[1] It has been reported that approximately 50% of all TMDs are myogenic in origin.[2] Myofacial pain of the masticatory muscles is more frequently induced by stress. In addition, it has been reported that parafunctional habits (i.e. clenching and grinding) is stress-related and replication of research forthe most common forms of muscle and joint-related disorders.[3]

Academic examinations are considered as one of the most acute stressors experienced by students. Acute stress has been reported to increase the activity of the hypothalamus-pituitary adrenal (HPA) axis with subsequent rise in cortisol level. [4, 5]

In the blood only 1 to 15% of cortisol is in its unbound or biologically active form. The remaining cortisol is bound to serum proteins.[6]

Unbound serum cortisol enters the saliva via intracellular mechanisms, and in saliva the majority of cortisol remains unbound to protein, because of partial conversion of cortisol to cortisone during passage through the salivary glands, the absolute level of free cortisol in saliva is 10% to 35% lower than it is in blood.[7]

The use of salivary biomarkers has gained increased popularity over the past decade in psychological and biomedical research since collecting samples is non-invasive and painless.[8] Salivary cortisol measurement is today a widely accepted as alternative to plasma or serum measurement, since. Salivary cortisol has been used as a measure of free circulating cortisol levels. In addition, the adrenal cortex is responsive to stress because venipuncture for blood collection can lead to an iatrogenic increase of plasma glucocorticoid levels.[9]The aim of this study was to evaluate the level of cortisol in saliva among sample ofUniversity students havingsymptom of myofacial pain during the final exam period and whether this finding could have a significant value as a psychobiological stress marker.

MATERIALS AND METHODS

The study was conducted in Baghdad University colleges. The study samples consist of ninety eight university students aged ranged between 18 to 30 years old. They were divided into two

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groups. The 1st group was fifty students with myofacial pain as a study group and the 2nd was forty eight students without myofacial pain as a control group. Myofacial pain evaluated according to RDC/TMD. The diagnosis of muscular disorders was basedon the anamnestic reports of pain in the muscles of mastication and clinical assessments of pain by palpation of at least three of twenty muscular sites in the facial area (ten for each side).[3]

Five ml of unstimulated salivary samples were collected from each student in the morning between 7-9am of the day of final examination before entering the exam and second sample were collected three months later for comparison. Measurement of salivary cortisol was done by means of High sensitivity, salivary cortisol enzyme immunoassay kit (Salimetrics Europe, Ltd.).

RESULTS

It has been shown that the mean level of salivary cortisol for the study group before exam was (1.988+0.068 µg/dl) and three months later was (0.377+0.245 µg/dl) whereas the mean for the control group before exam was (1.985+0.060 µg/dl) and after three months later was (0.416+0.234 µg/dl) as shown in table (1).

Testing the similarity between the concentration of salivary cortisol for the study and control groups in the before exam period has shown a non-significant results ($p>0.05$), as shown in table (2). While testing the alteration in the concentration of salivary cortisol between the two periods (before exam and three months later) for each group has shown a highly significant result ($p<0.001$), as shown in table (3).

The correlation between salivary cortisol concentration and pain is shown in table (4). The correlation coefficient between the concentration of salivary cortisol and pain by scoring (improvement) was ($r = -0.353$) with significant association at $p<0.05$ ($p=0.021$), which indicating that with increasing the concentration grade, decreasing with scoring pain also the correlation coefficient for concentration-differences (before & after) and pain scoring differences was negative (-0.245) and significant at $p<0.05$ (0.043) as shown in table (5) and figure (1).

DISCUSSION

The students perceive a high level of stress before the final written examinations. The authors were thought to utilize this period as stressful factor to design and conductthis trial. Myofacial pain is a symptom usually precipitated by stress and is usually noticed aggravated during the exams period among the University students. It has been proven that stress exaggerated cortisol response; therefore saliva cortisol level was used as a biomarker to determine the myofacial pain precipitated by stress symptom.

In thistrial the level of salivary cortisol, which was used as a marker of stress, was found to be non-significant between the study and control groups in the before exam period. However, there was a highly significant difference in both the study and control groups before the exam period compared with its level three months later. This finding is in consistent with other reported studies [8, 10,11]. The acute stress has been reported to increase the activity of the hypothalamus-pituitary adrenal (HPA) axis. The activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent release of cortisol are major components of the physiological stress response. Salivary cortisol accurately reflects serum cortisol, the physiologically active component.[4]While this findingdisagreed with Loft et al. and Takatsuji et al. who suggested that salivary cortisol may not be sensitive to the examination stressor.[12, 13]

In the present study, there was a highly significant differencein the degree orreduction in severity of pain between the two periods. This may be interpreted by the removal of stressor and decreasing of the parafunctional activities. Although some participants still had pain but with less score, this may be explained due to stressors of their social lifewhich is in agreement with Suvinen et al. who suggested that patients with TMDs often have onset of their symptoms during periods of psychological stress (i.e., anxiety) and exacerbation of symptoms during periods of stressful situations.[14]

As mentioned before the results revealed a high significant difference in salivary cortisol level between the two periods for the study and control group, but a non-significant difference between the two groups in before final examination period.A negative significant association was observed in the study between the concentration of salivary cortisol and pain, this result may be explained by the fight or flight response, was identified by Cannon, which is the physiological changes that prepared the body to acute stressor either physical or psychological.[15] The stress response is mediated by the activation of both the sympathetic nervous system and the hypothalamic-adrenal-pituitary axis. Many hormones are released, cortisol is one of them which have many functions, and one of them is

the shutting down of the initial fight or flight responses of the sympathetic nervous and immune systems to prevent them from overshooting and damaging the organism.[16]

During the stress response, both the brain and the pituitary gland release opiates such as endorphins and enkephalins which limit pain perception and their initial function may be primarily to inhibit or modulate the release of cortisol.[17, 18]

Although there is strong evidence that some TMDs patients are characterized by higher levels of general anxiety, compared with asymptomatic controls, the influence of stress on TMDs is probably not as simple as suggested according to Laskin's theory, in which stress evokes chronic recurrent muscular hyperactivity. and research findings have supported a relationship between anxiety, muscular tension, and TMDs symptoms.[19]The result showed that salivary cortisol level was reduced between the stressful or exam period and three months later, however the level was not different between the myofacial (symptomatic) and control (asymptomatic) group, therefore, this study concluded that salivary cortisol level may be used as a stress marker but not at the level as a stress related myofacial pain symptom indicator.

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Table 1. Predicated statistics of Concentration of salivary Cortisol

Sample	Period	No.	Mean	S.D.	S.E.
Study	**Before**	50	1.988	0.068	0.010
	After	50	0.377	0.245	0.035
Control	**Before**	48	1.985	0.060	0.009
	After	48	0.416	0.234	0.034

Table 2. Testing of similarity between the two independent groups (study and control) at the predicated concentration of salivary cortisol parameter in the before exam period of time

Parameter	Levene's Test for Equality of Variances		t-test for Equality of Means			
	F	Sig.	t	d.f.	Sig. (2-tailed)	C.S.
Concentration	0.036	0.851	0.232	96	0. 817	NS

Table 3. Testing of improvement between the two dependent periods (Before – After) for each group (study and control) for the predicated concentration of salivary cortisol parameter

Wilcoxon Signed Ranks Test		
After – Before	Study	Control
Z-test	-6.154	-6.031
Asymp. Sig. (2-tailed)	0.000	0.000
C.S.	HS	HS

Table 4. The correlation between the concentration of salivary cortisol and pain scoring

Correlation between salivary cortisol and pain scoring	Correlation coefficient	P-value
	-0.353	0.021

Table 5. Linear Person's correlation coefficient for concentration-differences (before & after) and pain scoring differences

Person Correlation Coefficient		Pain - Diff.	C.S.(*)
Pain - Diff.	Correlation	-0.245	S
	Sig. (1-tailed)	0.043	

(*) Sig. at P<0.05

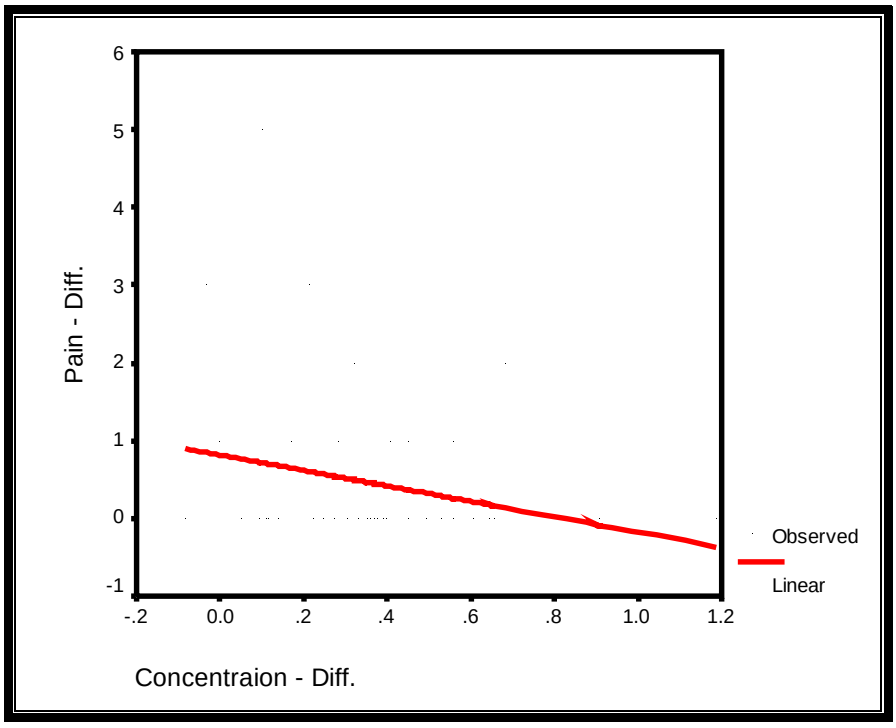

Figure 1. Linear Plot for Concentration-differences (before & after) and Pain scoring differences