Assessment of cortisol as salivary psychological stress marker in relation to temporomandibular disorders among a sample of dental students

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ABSTARCT

Background: University dental students perceived a higher level of stress prior to the final exam associated with raised salivary cortisol levels which could be considered as a useful noninvasive biomarker for measuring acute stress. Using a Helkimo anamnestic and clinical dysfunction scoring for temporomandibular disorders can give a better insight about the association of this marker and temporomandibular joint disorders. The aim of this study was to evaluation level of salivary cortisol in stressor students with temporomandibular disorder and the relation between this marker in relation to temporomandibular disorder severity. This might give a better understanding to the role of psychological stress as an etiological factor for developing temporomandibular joint problems.

Materials and methods: A total eighty participants age between 20 to 24 were recruited for this study. The participants were University dental students under graduate students at final examination period who were examined and gave saliva samples in final examination period. Salivary assay kits as cortisol was used to measure those variables and a Helkimo anamnestic and clinical dysfunction scoring for TMD.

Results: The group of participants with stress and temporomandibular disorder showed significantly higher levels of salivary cortisol than the control group, the salivary cortisol has statistically significant correlation with Helkimo anamnestic categories (Di-I mild, Di-II moderate and Di-III severe. Salivary cortisol levels show significant but weak association with two categories of clinical dysfunction criteria in Helkimo index system, which are Muscle pain and TMJ pain on palpation.

Conclusion: This study demonstrated that University students perceived a high level of stress before the final examination. Salivary cortisol is the stress biomarker that is most often used to measure acute stress. Helkimo anamnestic and clinical dysfunction scoring criteria for still the pioneer for measuring a temporomandibular disorder.

Keywords: stress, cortisol, Helkimo, temporomandibular joint TMJ, temporomandibular disorder TMD. (J Bagh Coll Dentistry 2015; 27(2):86-92).

INTRODUCTION

University students are liable to a higher level of stress especially in pre-examination period, if stress is prolonged, the stress response has two principal facets: the neuro-endocrine, which involves corticotropin-release hormone, activation of the hypothalamic-pituitary-adrenal axis and the secretion of cortisol into circulation. Cortisol is then filtered through the acinar cell membrane of the salivary glands, and is found in saliva in the free unbound form. Secondly, the stress response involves activation of the autonomic nervous system, release of catecholamines (e.g., plasma norepinephrine, pNE), and sympatho-mimetic manifestations, such as increase salivation, and increase secretion of Sα-amylase [(1)]. Salivary cortisol levels increase under a variety of physical (i.e., exercise, heat and cold) and psychological (i.e., written examinations) challenges. Salivary and plasma cortisol levels always correlate with each other following stress, confirming that the two pathways are the same salivary cortisol increases with psychological stress and correlates serum cortisol [(2)].

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Temporomandibular joint dysfunction is an collective term covering pain and dysfunction of the muscles of mastication and the temporomandibular joints. The most important feature is pain, .followed by restricted mandibular movement and noises from the temporomandibular joints (TMJ) during jaw movement [(3)]. Although temporomandibular disorder TMD is not life threatening, it can be detrimental to quality of life [(4)] because the symptoms can become chronic and difficult to manage. Usually people affected by TMD are between 20 and 40 years of age, and it is more common in females than males [(5)].

The etiology of TMDs is multifactorial which is thought to be caused by multiple, poorly understood factors [(6)]. But the exact etiology is unknown [(7)]. There are factors which appear to predispose to TMD (genetic, hormonal, anatomical), factors which may precipitate it (trauma, occlusal changes, parafunction), and also factors which may prolong it (stress and again parafunction) [(8)]. Overall, 2 hypotheses have dominated research into the causes of TMD, namely a psychosocial model and a theory of occlusal disharmony [(7)].

Oral habits or parafunctions, defined as any oral nonfunctional activity or behaviour involving

the masticatory system, are neither uncommon nor are they always harmful [9]. It is only when such activities exceed an individual's physiologic tolerance that breakdown of the masticatory system may occur. In such cases the initial breakdown takes place in the tissue with the lowest structural tolerance in that particular individual, e.g. joints, teeth or muscles [10]. Oral habits or parafunctions have been reported to be common worldwide, with many students and adolescents performing them on a daily basis [11]. Oral habits include a variety of activities, such as continuous gum chewing, nail biting, and chewing on writing implements (pencils, pens).

Oral habits such as these are common among students, and they were shown to have a potentially detrimental effect on the masticatory system [12,13]. Saliva has been described as the mirror of the body. The wide spectrum of compounds present in saliva may provide information for clinical diagnostic applications. Saliva is a good medium because its collection is noninvasive and the donation process is relatively stress free, so that multiple collections can be performed without imposing too much discomfort on the donor [14]. Cortisol, known more formally as hydrocortisone is a steroid hormone, more specifically a glucocorticoid, produced by the zona fasciculata of the adrenal cortex [15]. It is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to increase blood sugar through gluconeogenesis; suppress the immune system; and aid in fat, protein and carbohydrate metabolism. It also decreases bone formation [16].

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 [17]. 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 [18]. Salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes [19].

SUBJECTS, MATERIALS AND METHODS

The subjects:

A total eighty participants age between 20 to 24 were recruited for this study. The participants were University dental students under graduate students at final examination period who were examined and gave saliva samples in final examination period. The participants in this study divided into two groups:

-Case group: sixty stressed students with temporomandibular disorders (TMD).

-Control group: twenty students without stress and temporomandibular disorders (TMD).

***Inclusion criteria**

1. University dental students (20-24) years old from both genders with stress and temporomandibular disorders were included in the case group. The female students were in the luteal phase of menstrual cycle to be equal to male in the activity of hypothalamus-pituitary-adrenal axis.
2. University dental students (18-30) years old from both genders without stress and temporomandibular disorders were included in the control group.

*** Exclusion criteria**

Students with a history of use of corticosteroids in the past year, a history of antidepressant medication or head injury, on hormone supplements including oral contraceptives at the time of saliva collection., orthodontic treatment, occlusal disharmonies like cross bite and premature contact or dental pain. Those with muscle tenderness due to systemic diseases as fibromyalgia, neuralgia and local infection and Cases with more than 2 missing posterior teeth.

Materials: High sensitivity, salivary cortisol enzyme immunoassay kits (Uscn Life Science Inc. Wuhan, China).

Methods of examination: The participants examined according to Helkimo anamnestic and clinical dysfunction index of temporomandibular disorders which consists of standardized series of diagnostic tests based on clinical signs and symptoms.

Statistical Analysis

Statistical analysis was computer aided. An expert statistical advice was sought for. Statistical analyses were done using IBMSPSS version 21 computer software (Statistical Package for Social Sciences). Data were presented in measures of mean, standard deviations, range (minimum-maximum values), median, frequency and percentages. The significance in difference between the means (quantitative data) for two groups was tested using independent student t-test, while using Analysis of Variance (ANOVA) for more than two groups the receiver operating

characteristics (ROC) and predictive value (PV) was used in this study. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. Probability test (P value) was considered statistically significant when the P value < 0.05 and regarded as highly statistically significant when the P value < 0.001.

Table 1: The salivary cortisol levels in the case and control groups.

Salivary cortisol	Study groups		P
	Controls	Cases with stress	<0.001
Range	(9.8 to 46)	(9.6 to 413)	
Mean	22.9	177.8	
SD	9.9	94.8	
SE	2.28	12.24	
N	20	60	

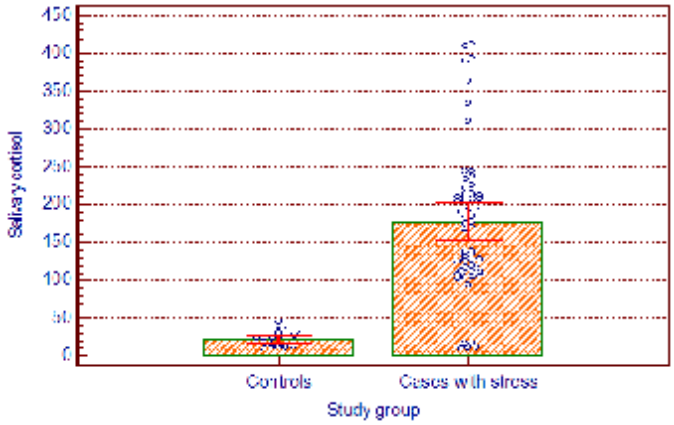

Figure 2: Dot diagram with error bar chart demonstrates the comparison of salivary cortisol levels in both control and stress with TMD groups. Bars represent the median while range lines represent the 95% Confidence Interval CI (measurements in ng/ml and P value< 0.001).

The predictive value measurements for salivary cortisol showed highly positive predictive value (PPV) at 50% and 90% levels with the cut-off point of 70.1 ng/ml. Above this point, the salivary cortisol can be used as biomarker for accurate prediction of stress and TMD (accuracy 93.7%).

Correlation analysis of salivary cortisol with Helkimo anamnestic clinical dysfunction score shows positive association expressed by significant p value of 0.026. This means that the salivary cortisol has statistically significant correlation with Helkimo anamnestic categories (Di-I mild, Di-II moderate and Di-III severe). See Table 2 and Figure 3.

RESULTS

The data related to salivary cortisol levels showed normal distribution in both case and control groups. The group of participants with stress and TMD showed significantly higher levels of salivary cortisol (mean 177.8 ± SE 12.24 ng/ml) than the control group (mean 22.9 ± SE 2.28 ng/ml). See Table 1 and Figure 2.

Table 2: Association of Salivary cortisol levels with Helkimo anamnestic categories of TMD

Salivary cortisol	Study groups			P
	(1–4) Di-I (Mild dysfunction)	(5–9) Di-II (Moderate dysfunction)	(10–25) Di-III (Severe dysfunction)	<0.001
Range	(9.6 to 410)	(105 to 413)	(185 to 364)	
Mean	158.8	221.9	264.7	
SD	81.3	117.6	91.1	
SE	12.26	32.63	52.6	
N	44	13	3	

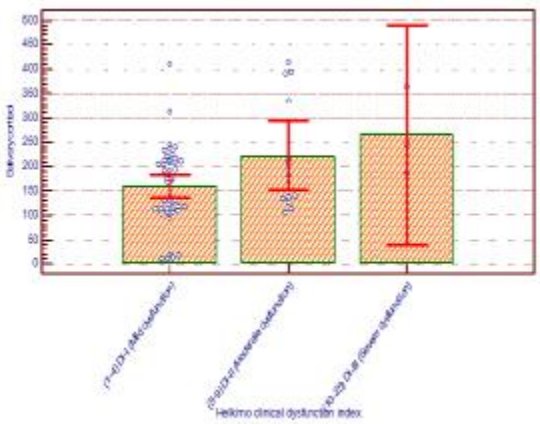

Fig 3: Dot diagram with error bar chart demonstrating the association of salivary cortisol levels with Helkimo anamnestic categories of TMD. Bars represent mean of the salivary cortisol in each category, range lines represent standard errors and p value <0.05.

In depth analysis with each single criteria used by Helkimo, revealed more interesting results. Salivary cortisol levels show significant but weak association with two categories of clinical dysfunction criteria in Helkimo index system, which are Muscle pain and TMJ pain on palpation. The muscle of mastication with highest tenderness to palpation was master muscle followed by temporalis and lateral pterygoid with less tenderness. See table 3. The distribution percentage of varying oral habits in TMD students that shown as follow (See Table 4).

Table 3: Association of salivary cortisol levels with individual clinical criteria used in Helkimo index for TMJ dysfunction

	Salivary cortisol					
	Range	**Mean**	**SD**	**SE**	**N**	**P**
Gender						
Female	(9.6 to 410)	191.1	94.2	17.19	30	0.28[NS]
Male	(11.2 to 413)	164.5	95.2	17.39	30	
Mandibular mobility (opening, laterotrusive, protrusive)						0.027
Normal range of movement	(9.6 to 394)	158.7	82.8	13.09	40	
Slightly impaired mobility	(105 to 413)	215.9	107.5	24.05	20	
Severely impaired mobility	**	**	**	**	0	
r=0.202 P=0.12[NS]						
Symptom: impaired TMJ function						0.01
Smooth movement without TMJ sounds and deviation on opening or closing movement <2 mm	(104.4 to 410)	219.2	85.9	21.47	16	
Sounds in 1 or both joints and/or deviation >2 mm on opening or closing movements	(9.6 to 413)	156	90.2	13.92	42	
Locking/and/or luxation of the TMJ	(245 to 364)	304.5	84.1	59.5	2	
r=-0.191 P=0.14[NS]						
Symptoms: Masseter pain						0.007
No tenderness to palpation in masticatory muscles	(9.6 to 212.2)	114.7	75.8	26.78	8	
Tenderness to palpation in 1-3 palpation sites	(11.2 to 413)	177.6	89.8	13.11	47	
Tenderness to palpation in > 4 palpation sites	(185 to 394)	280.6	92.9	41.55	5	
r=0.373 P=0.003						
Symptoms: TMJ pain						0.045
No tenderness to palpation	(9.6 to 410)	154.5	96.3	17.89	29	
Tenderness to palpation laterally	(16.4 to 413)	199.5	89.5	16.08	31	
Tenderness to palpation posteriorly	**	**	**	**	0	
r=0.286 P=0.027						
Symptom: pain on movement of the mandible						0.09[NS]
No Pain on movement	(9.6 to 240)	162.9	68.1	14.53	22	
Pain on 1 movement	(11.5 to 413)	174.5	103.4	18	33	
Pain on > 2 movements	(139 to 390)	264.6	109.7	49.04	5	
r=0.101 P=0.44[NS]						

Table 4: The distribution percentage of varying oral habits in TMD students.

Oral habits	N	%
Non	12	20.0
Clenching	17	28.3
Grinding	5	8.3
Biting nail	4	6.7
Bruxism	13	21.7
Chewing	9	15.0
Total	60	100.0

DISCUSSION

For decades, research on the acute and chronic effects of stress has employed cortisol levels as an index of the individual response to stress [20]. Salivary cortisol levels provide an accurate, reliable, and non-invasive measure of stress in both adults and children [21]. Which match the

result of this study that showed salivary cortisol is useful biomarker for assess psychological stress. Cortisol is a hormone secreted by the hypothalamus pituitary adrenal axis (HPAA) and has been used as an accurate biomarker in stress research for over half a century [22]. In dentistry salivary cortisol has been used to measure the role of stress in a variety of settings from the anxiety of dental treatment to periodontal disease, dental caries and temporomandibular disorders [23-26]. As already known, cortisol secretion increases significantly in the state of acute stress as in psychological stressor of written examinations [20, 27, 28]. These results have an agreement with this study that have found a highly significant levels of salivary cortisol which in turn strongly support these previous studies that have showed and suggested the use of cortisol as non-invasive indicator for assessment stress with TMD.

In 2004, Dickerson and Kemeny had found that the peak cortisol response occurs between 21 and 30 minutes from the onset of the examination, and that it does not depend on the duration of the examination [27]. Salivary cortisol levels increase several fold within a short time period after the onset of psychological stress [29], and those results have an agreement with this study while disagree with [30-34], which showed that the results related to written examinations (tests) were heterogeneous and not necessarily accompanied by cortisol increase.

The predictive value measurements for salivary cortisol showed highly positive predictive value (PPV) at 50% and 90% levels with the cut-off point of 70.1 ng/ml. Above this point, the salivary cortisol can be used as biomarker for accurate prediction of stress and TMD (accuracy 93.7%).

Temporomandibular joint disorder (TMD) represents a common health problem [35]. It is an umbrella term embracing a number of clinical manifestations that involve the temporomandibular joint (TMJ), the masticatory muscles and the teeth. Patients with TMD usually suffer from muscle and/or joint pain on palpation and on mandibular movements, joint sounds and the mandibular range of motion may be limited [36]. TMD can affect any patients regardless of age including children [37]. Or gender with varying signs and symptoms [38]. These investigations are in agreement with the result of this study that have found there is no significant difference between age and gender groups for both case groups (stress with TMD) and control without TMD problems, Statistical analysis showed the association of salivary cortisol with the clinical criteria of TMJ that involve pain of soft tissue (muscles and TMJ ligaments), while the other clinical criteria (impaired mobility, impaired TMJ function and painful movement of mandible) did not show any associated with salivary cortisol. This indicate that acute stress causing higher level of cortisol which have greater impact on the early clinical criteria such as muscle pain and TMJ pain and the other criteria that have established mobility impairment may not related to acute stress thus show no association. These other criteria may be related to chronic stress rather than acute type. TMDs can be subdivided into muscular and articular categories. Differentiation between the two is sometimes difficult because muscle disorders may mimic articular disorders, and they may coexist [39]. In the present study it was used Helkimo anamnestic and clinical dysfunction index as a scoring for TMD students and the results that the muscles have pain on palpation as following: Masseter muscles (40%), Temporalis (31%), lateral pterygoid (9%), medial pterygoid (6%), anterior diagastric (4%) and Sternocleidomastoid (2%), while the students with no symptoms of myofascial pain (8%). Parafunctional habits of masticatory muscles, with and without associated chronic pain. In this study the distribution percentage of varying oral habits in TMD students that shown as follow: Clenching (28.3%), Bruxism (21.7%),Chewing: lip, gum, pen, cheek (15%), Grinding (8.3%), Bitting nail (6.7%), while those have no parafunctions habits was (20%). It is believed that habits can act as an important etiologic factor of TMD, as they lead to a traumatic dental occlusion that may affect the teeth and the masticatory muscles and temporo-mandibular joints, causing the disruption of the functional balance stomatognathic system, or worsening the already installed TMD [12, 40].

This study demonstrated that salivary cortisol can be used as a stress predictive biomarker to assess the severity of TMJ problems due to psychological stress in university students.

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الخلاصة

الخلفية : ان طلبة كلية طب الاسنان يتعرضون الى مستويات عالية من التوتر النفسي قبل الامتحانات النهائية .نتيجة لذلك نشأت حاجة ماسة لمؤشر حيوي غير تداخلي لقياس التوتر الحاد عند طلبة الجامعة .هنالك عدة دراسات اعتبرت هرمون الكورتيزول اللعابي كمؤشر حيوي لقياس التوتر .أن استخدام نظام هلكيمو لتسجيل مدى الاختلال الوظيفي السريري للمفصل الصدغي ممكن ان يعطي تصور جيد عن العلاقة بين هذا المؤشر الحيوي واختلال المفصل الصدغي الوظيفي السريري.

اهداف الدراسة :هو تقييم مستوى هرمون الكورتيزول اللعابي عند طلبة الجامعة عند تعرضهم للتوتر واللذين يعانون من اختلال المفصل الصدغي الوظيفي .وهذا ربما يعطينا فهم افضل لدور التوتر النفسي كعامل مسبب لاختلال الوظيفي السريري لمفصل الصدغي.

المواد وطريقة العمل : تم انجاز هذه الدراسة على مجموعة متكونة من ثمانين من طلبة كلية طب الاسنان جامعة بغداد اللذين تتراوح اعمارهم بين عشرين الى اربعة وعشرين عاما .كان منهم ستون طالبا يعانون من اختلال وظيفي سريري في المفصل الصدغي اختيروا كمجموعة الدراسة .أما العشرون البقية فكانوا سريريا بدون اي اختلال وظيفي في المفصل الصدغي واللذين اختيروا كمجموعة المراقبة .وقد تم فحص المشاركين وفقا لمعايير البحث وتشخيص اضطرابات واختلال المفصل الصدغي حسب نظام هلكيمو .تم جمع خمسة ملي لترات من عينات اللعاب الغير المحفز قبل الامتحان النهائي لقياس تركيز الاميليز والكورتيزول اللعابيين.

النتائج : لقد اظهرت النتائج علاقة طردية بين مستوى هرمون الكورتيزول و الاختلال الوظيفي للمفصل الصدغي للمشاركين فيالدراسة.

استنتاج : نستنتج من هذه الدراسة امكانية استخدام هرمون الكورتيزول كمؤشر حيوي لقياس التوتر النفسي عند طلبة الجامعة وعلاقته بالاختلال الوظيفي السريري للمفصل الصدغي.