An Assessment of Alpha-Amylase 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 alpha-amylase 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 disorders. The aim of this study was to evaluation level of salivary alpha-amylase in stressor students with temporomandibular disorders and the relation between the marker in relation to temporomandibular disorders 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 aged 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 alpha-amylase was used to measure those variable and a Helkimo anamnestic and clinical dysfunction scoring for temporomandibular disorders.

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

Conclusion: This study concluded that University students perceived a high level of stress before the final examination. Salivary alpha-amylase is now 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 TMD.

Keywords: Stress, alpha-amylase, Helkimo, temporomandibular joint, temporomandibular disorders. (J Bagh Coll Dentistry 2015; 27(4):90-95).

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 Salivary alpha-amylase. Salivary alpha-amylase levels increase under a variety of physical (i.e., exercise, heat and cold) and psychological (i.e., written examinations) challenges. Salivary and plasma norepinephrine levels always correlate with each other following stress, confirming that the two pathways are the same [1].

Temporomandibular joint dysfunction is a 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 [2]. Although temporomandibular disorders (TMD) are not life threatening, it can be detrimental to quality of life [3] 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 [4].

The etiology of TMDs is multifactorial, which is thought to be caused by multiple, poorly understood factors [5]. But the exact etiology is unknown [6]. 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) [7]. Overall, 2 hypotheses have dominated research into the causes of TMD, namely a psychosocial model and a theory of occlusal disharmony [6].

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Oral habits or parafunctions, defined as any oral nonfunctional activity or behaviour involving the masticatory system, are neither uncommon nor are they always harmful [8]. 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 [9]. Oral habits or parafunctions have been reported to be common worldwide, with many students and adolescents performing them on a daily basis [10]. 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 [11,12].

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 [13].

Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion [14]. Salivary α-amylase has been used as a biomarker for stress that does not require a blood draw [15].

Recently, much attention has been given to possible interactions between stress and α-amylase levels. However, significant psychosocial studies of α-amylase responsiveness are difficult due to the system's complexities. Salivary α-amylase (sAA) is secreted by the parotid gland in response to adrenergic activity and is suppressed by β-adrenoreceptor blockade [16]. It has also become established as a new biomarker of the psychosocial stress response within the SAM system [17]. Studies show marked increases in sAA levels in response to stressful tasks or procedures, such as a parachute jump or a stressful video game, as well as to other types of psychological (e.g. pre-examination) stressors [18].

MATERIALS AND METHODS

The Subjects:

A total 80 participants age between 20 to 24 years were recruited for the present 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:

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

Materials:

High sensitivity, salivary alpha-amylase 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, percentages and predictive value. 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. Different percentages (qualitative data) were tested using chi-square test, Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. 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.

RESULTS

The data related to salivary alpha-amylase levels showed skewed distribution in case groups. Therefore, comparison between case and control groups has been made using median and interquartile range to overcome the skewed data distribution. The group of participants with stress and TMD showed significantly higher levels of salivary amylase (median 494 with IQR 339-1016 ng/ml) than the control group (median 270 with IQR of 89-313 ng/ml) as shown in table 1.

The predictive value measurements for salivary alpha-amylase showed highly positive predictive value (PPV) at 50% and 90% levels with the cut-off point of (314) ng/ml. This point can be used as biomarker reference value for the salivary alpha-amylase for accurate prediction of stress and TMD (accuracy 84.8%).

Statistical analysis did not show any correlation of salivary alpha-amylase with Helkimo clinical dysfunction score of TMJ expressed by non- significant p value of 0.85 and approximately similar medians (500, 488, and 499 ng/ml) among all categories of Helkimo anamnestic categories (Di-I, Di-II and Di-III) as shown in table 2.

Association analysis of salivary alpha amylase level with clinical criteria of Helkimo clinical dysfunction index shows no association with any criteria whether mobility related or pain related. The p value was non-significant throughout all the clinical criteria categories as seen in table 3.

The distribution percentage of varying oral habits in TMD students was shown in table 4.

Table (1): The Salivary Alpha-Amylase Levels in the Case and Control Groups. (Measurements are in ng/ml and p<0.001).

Salivary alpha amylase (U/ml)	Study groups		p
	Control	Cases with stress	
Range	26-489	217-1992	<0.001
Median	270	494	
Inter-quartile range	89-313	339-1016	
N	19	60	
Mean rank	15.5	47.8	

Table (2): Association of Salivary Alpha Amylase Levels with Helkimo Anamnestic Categories of TMD.

Salivary alpha amylase (U/ml)	Helkimo clinical dysfunction index			P
	(1–4) Di-I (Mild dysfunction)	(5–9) Di-II (Moderate dysfunction)	(10–25) Di-III (Severe dysfunction)	
Range	217-1992	287-1854	363-591	0.85 (NS)
Median	500	488	499	
Inter-quartile range	319-1016	385-1173	363-591	
N	44	13	3	
Mean rank	29.9	32.8	28.7	
r=0.021, p=0.87 (NS)				

Table (3): Association of Salivary Amylase Levels with Individual Clinical Criteria Used in Helkimo Index for TMJ Dysfunction.

	Salivary amylase (U/ml)					
	Range	**Median**	**Inter-quartile range**	**N**	**Mean rank**	**P**
Gender						0.83 (NS)
Female	287-1920	510	361-672	30	30	
Male	217-1992	442	320-1438	30	31	
Mandibular mobility (opening, laterotrusive, protrusive)						0.84 (NS)
Normal range of movement	217-1992	500	319-1016	40	30.2	
Slightly impaired mobility	281-1854	494	352-918	20	31.2	
Severely impaired mobility	**	**	**	0	**	
r=0.027 P=0.84[NS]						
Symptom: impaired TMJ function						0.73 (NS)
Smooth movement without TMJ sounds and deviation on opening or closing movement <2 mm	315-1992	490	354-1576	16	33.4	
Sounds in 1 or both joints and/or deviation >2 mm on opening or closing movements	217-1891	494	323-983	42	29.5	
Locking/and/or luxation of the TMJ	363-591	477	363-591	2	27.5	
r=-0.104 P=0.43[NS]						
Symptoms: Masseter pain						0.68 (NS)
No tenderness to palpation in masticatory muscles	285-1048	554	347-804	8	29.2	
Tenderness to palpation in 1-3 palpation sites	217-1992	488	331-1245	47	31.4	
Tenderness to palpation in > 4 palpation sites	323-591	385	363-499	5	24.4	
r=-0.039 P=0.77 (NS)						
Symptoms: TMJ pain						0.89 (NS)
No tenderness to palpation	217-1992	536	320-983	29	30.2	
Tenderness to palpation laterally	281-1891	488	352-1173	31	30.8	
Tenderness to palpation posteriorly	**	**	**	0	**	
r=0.018, P=0.89 (NS)						
Symptom: pain on movement of the mandible						0.57 (NS)
No Pain on movement	256-1992	382	299-1438	22	27.5	
Pain on 1 movement	217-1891	488	385-983	33	32	
Pain on > 2 movements	363-1228	591	499-598	5	34.2	
r=0.138 P=0.29[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

Educational literature has reported the experience of stress in students and provides evidence that stress impairs academic performance[19-21]. Previous investigations analyzing physiologic responses to stress and performance have examined changes in heart rate, blood pressure, the presence of perspiration, and plasma catecholamine levels [15,22,23].

The measurement of salivary alpha-amylase, a valid and highly reliable method to measure the physiologic response to acute stress, has been incorporated into a multitude of biobehavioral research studies [15,24]. Salivary alpha-amylase is a major secretory protein found in saliva and aids in the initial digestion of starch [25,26]. Release of salivary alpha-amylase is regulated by autonomic innervation [17].

Sympathetic stimulation causes high salivary α-amylase release from the parotid and submandibular acinar cells, whereas parasympathetic stimulation causes low salivary α-amylase release from the sublingual acinar cells [25]. Recent investigations in academic students before examinations have demonstrated that salivary alpha-amylase levels significantly increase as a result of acute stress [23,24,27-29]. These recent investigations have an agreement with this study which concluded that there is a highly significant levels of salivary alpha-amylase in stress group with TMD than control one.

In 1997 Chatterton and colleagues linked levels of salivary α- amylase to sympathetic activation during physically and psychologically stressful conditions [30]. For purpose of the present study it was found that the level of salivary α-amylase was observed to increase significantly in an investigation that used university students before the written examinations as a psychological stressor.

Despite the skewed data of salivary alpha-amylase in case group due to small sample size, the comparison between the case and control group using the median and Inter-quartile range (IQR) showed the highly significant evidence of possible use of salivary alpha-amylase as alternative indicator or in conjunction with cortisol as biomarkers for assessing the stress with TMD [31].

The predictive value measurements for salivary alpha-amylase showed highly positive predictive value (PPV) at 50% and 90% levels with the cut-off point of (314) ng/ml. This point can be used as biomarker reference value for the salivary alpha-amylase for accurate prediction of stress and TMD (accuracy 84.8%).

Association analysis of salivary alpha amylase level with clinical criteria of Helkimo clinical dysfunction index shows no association with any criteria whether mobility related or pain related. The p value was non-significant throughout all the clinical criteria categories. Therefore the higher levels of salivary alpha-amylase in TMD indicating that participants have a problem with stress or TMJ problems but this biomarker have no any association neither severity nor clinical dysfunction criteria of Helkimo.

As conclusion; the present study demonstrated that salivary alpha-amylase can be used as a stress predictive biomarker to assess the TMJ problems due to psychological stress in university students.

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