

Evaluation of Salivary Cortisol and IgA levels in Stress Related Oro-facial Conditions

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ABSTRACT

Background: some people had special problems causing them to have a stress and university dental students perceived a stress with certain examinations. So, there was a need for measuring the stress level in those participants by using useful biomarkers. Several studies considered cortisol as a biomarker of stress with other biomarkers that were used in conjunction. By clinical diagnosis and the appearance of the lesions, the stress related orofacial conditions were determined.

Aim of the study: The aim of the study is to estimate and compare salivary (cortisol and IgA) levels in stress related orofacial conditions and normal people. Also, the differences between these markers in relation to these conditions. This could give an understanding to the role of stress in developing Oro-facial conditions.

Subjects, materials, and methods: The participants included in this study were undergraduate students in Baghdad and Babylon University who gave saliva samples at the period of academic examinations and some of other stressed people. ELISA kits (cortisol and IgA) were used to measure the variables in the saliva samples. The clinical diagnosis was depended in this study.

Results: The general group of stress showed a high significant level of salivary cortisol in comparison with the control group. According to patients' subgroups, there was a highly significant difference in the salivary cortisol level recorded with (traumatic cheek biting, and RAS) subgroups, a significant difference with tongue indentation subgroup, while a non-significant difference with both of (TMJDs and herpes labialis) subgroups in comparison with controls. Considering salivary IgA, there was a non-significant difference of salivary IgA level in the general patient's group considering control group. According to the subgroups, there was a highly significant difference between stressed patients with herpes labialis and controls, whereas there was a non-significant difference with the other comparisons.

Conclusion: This study shows that salivary cortisol can be used as stress biomarker to assess stress level in persons with orofacial conditions more than salivary IgA.

Key words: Stress, Salivary cortisol, Salivary IgA

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INTRODUCTION

Stress is a mental, physical, or emotional response to events that cause mental or bodily tension [1]. During stress, human body sustains a series of chemical events that affect multiple organs giving us the capability for bearing these repulsive stimuli. The body undergoes various homeostatic/ endocrinal/ immunological / metabolic/ disturbances due to continued exposure to the chemical changes. Common examples are hypertension, diabetes mellitus, headache, gastric ulcerations, etc. [2]. A psychosomatic disorder involves both the mind and body because they influence each other. The diseases interact between mind and body and body and mind [3]. Mouth is

concerned with major human passion directly or symbolically.

Oral diseases with psychosomatic cause have been known in medicine. Emotional or mental factors can act as risk factors which might affect the initiation of oromucosal diseases [4,5].

stressed People could establish parafunctional habits, i.e, unconscious efforts to decrease stress ex: chewing, biting on pencil or other objects that lead to abnormal positioning, dental attrition and closure of the jaws tightly effecting the muscles of mastication that lead to bruxism and myofascial pain dysfunction syndrome (MPDS)-like symptoms. Irregular eating habits with gastric disturbances can induce vomiting causing dental erosion [6].

Other oral problems which begin because of stress include: aphthous ulcers, burning mouth syndrome,

xerostomia, oral lichen planus, bruxism and MPDS [7]. Stress is considered as a risk factor for lichen planus which is an autoimmune disease [1]. Psychological stress is a stimulating factor for (RAS), and it is found that the lesions occur in stressful situations [8] like dental treatment, school exams, and periods of considerable changes in life. Behavioral/psychiatric disorders and sleep disturbances are common causative factors of bruxism. Burning mouth syndrome is commonly present in persons with behavioral or psychogenic disorder and sleep disturbances [1]. Xerostomia also known as dry mouth, which is the abnormal salivary reduction, a common find in psychiatric patients [9].

Cortisol is a steroid hormone, within glucocorticoid class of hormones. It is made in the cortex of the adrenal glands and then released into the blood in response to stress and low blood-glucose concentration [10], and then the blood transports it all round the body. It acts to elevate blood sugar by process of gluconeogenesis, to inhibit the immune system, also, to aid in metabolism of carbohydrates, fat, and protein [11].

Cortisol is secreted in a circadian rhythm, levels rise at 02:00-04:00 a.m., then it reaches to peak after awaking (CAR: cortisol awakening response) then decrease during daytime until reach the lowest level at midnight [12].

The concentration of salivary cortisol accounts for 70% of unbound blood cortisol because of its lip solubility and low molecular weight, by free diffusion way, passes across the basal membrane of salivary glands acinar cells into saliva. Several studies have determined that the analysis of salivary cortisol as a stress biomarker is a reliable alternate to blood and urinalyses [13-15].

Immunoglobulin A (IgA) also called as sIgA in its secretory form. It is an antibody which plays an important role in the immunity of mucous membranes. Its amount produced in relation with mucosal membranes is larger than other types of antibodies combined [16]. It is noted that acute stress can decrease the efficiency of the immune system and inhibit the production of immunoglobulin [17]. IgA has two subdivisions (IgA1 and IgA2) and can be produced in both forms as a monomeric and as a dimeric. The most prevalent is the dimeric form also named secretory IgA (sIgA) which is the main type of immunoglobulins present in mucous secretions, involving tears, colostrum, sweat, saliva and secretions from the gastrointestinal tract, genitourinary tract, respiratory epithelium, and prostate. Also, it is found in small concentrations in blood. The secretory piece of sIgA protects it from being degraded by the proteolytic enzymes; therefore sIgA can survive in the harsh environment of gastrointestinal tract and provide protection against the microbes which multiply in body secretions [18]. sIgA can also suppress inflammatory effect of other immunoglobulins [19, 20] have studied the difference in the experience of stress in dental students in junior year, before and after exam, measuring the cortisol levels, chromogranin A (Cg A) and secretory IgA in saliva. The results indicated that a higher score of stress was recorded before the exams in

comparison to the values taken after the exams ($p=0.015$), levels of cortisol were higher before the exams ($p=0.015$), whereas the differences concerning the values of CgA and secretory IgA in saliva before and after the exams were not statistically confirmed.

MATERIALS AND METHODS

The study was performed in the university of Baghdad and Babylon/college of dentistry who gave salivary samples at time of examination and some of other people who exposed to stress in the period between December/2018-March/2019. The samples were collected from participants suffering from stress related oro-facial conditions. Clinical examination and information were collected using case sheets. The participants were consented if they agree to take part in the study and if didn't, it would not affect the treatment.

Inclusion criteria

Persons who are diagnosed with emotional stress and having stress related orofacial conditions such as traumatic cheek biting, tongue indentation, herpes labialis, recurrent aphthous ulceration (RAS) and temporomandibular joint disorders (TMJDs).

Exclusion criteria

Patients are excluded if they are:

- On medications that cause eruption of oral lesions
- Those with malocclusion that causes ulceration or TMJ problem.
- Pregnant women.
- Those with systemic diseases apart from stress.
- Persons who don't give their consent form.

The samples of saliva were collected then centrifuged at 2500 rpm for 15 min. The ELISA kit was used. The competitive-ELISA principle was used for evaluation the salivary cortisol level. While sandwich-ELISA method was used for salivary IgA evaluation.

RESULTS

A total of 107 patients diagnosed with stress (102 females, 6 males) and 30 volunteers (28 females, 2 males) participated in this study. The mean age was 23.94 ± 6.14 years for the study group and 30.10 ± 9.60 years for controls. The gender difference between the two groups was non-significant.

The concentrations of both markers were measured in saliva using enzyme linked immunosorbent assay (ELISA) kit. Analysis of salivary cortisol was done by competitive-ELISA principle, while sIgA by sandwich-ELISA principle.

The results have shown that tests distributions are non-normal generally. Table 1 shows Mann-Whitney-U test statistics for testing combinations among different studied groups in each marker. Significant level was recorded at P -value=0.05, and highly significant at

P<0.01. The combinations are accounted independently. There is a highly significant level of salivary cortisol in two groups of patients (cheek biting and RAS) in comparison with control group. Also, with reference to real significant level concerning tongue indentation group (P= 0.06), this relationship can be reported as a significant statistically. While there is a non-significant difference between the other case groups of (herpes labialis and TMJDS) and controls. According to SigA level, the table shows a highly significant difference between case group of herpes labialis and control group, while

there is a non-significant difference with the other comparisons. Table 2 shows an estimation area of ROC curve for morbidity indicators in admixed all diseases groups regarding studied markers in comparison with controlled groups. The results show a meaning differentiation was assigned within "cortisol" marker, because a positive high significant area is accounted at P<0.01, also a meaning differentiation was assigned within "melatonin" marker, because a positive significant area is accounted at P<0.05.

Table 1: Mann-whitney-U test.

Combination	Groups	Cortisol	IgA
	Mann-Whitney U test		
Control X cheek biting	Z-value	-3.524	-1.302
	Asymp. Sig. (2-tailed)	0	0.193
	P-value	0	2.895
	C.S.	HS	NS
Control X RAS	Z-value	-3.392	-1.667
	Asymp. Sig. (2-tailed)	0.001	0.095
	P-value	0.015	1.425
	C.S.	HS	NS
Control X herpes labialis	Z-value	-0.096	-3.947
	Asymp. Sig. (2-tailed)	0.924	0
	P-value	13.86	0
	C.S.	NS	HS
Control X tongue indentation	Z-value	-2.854	-1.724
	Asymp. Sig. (2-tailed)	0.004	0.085
	P-value	0.06	1.275
	C.S.	S	NS
control X TMJDs	Z-value	-1.562	-0.646
	Asymp. Sig. (2-tailed)	0.118	0.518
	P-value	1.77	7.77
	C.S.	NS	NS

Asymp. Sig.=Asymptotic Significance; C.S.=comparison significant;
 NS: Non significant at P>0.05; S: Significant at P<0.05; HS : Highly significant at P<0.01

Table 2: ROC curve for morbidity indicators in admixture formed for all diseases groups regarding studied markers.

Diseases & markers	Cutoff Point	Sensitivity	Specificity	Area	Std. Error	Asy.Sig. (*)	Asy. 95% C.I.		
							L.b.	U.b.	
All Diseases	Cortisol	253.19	0.372	0.84	0.726	0.06	0.001	0.608	0.843

IgA	493.21	0.479	0.24	0.208	0.063	0.179	0.288	0
								5
								3
								7

(*) HS: Highly Sig. at P<0.01; (*) S: Sig. at P<0.05; Non-Sig. at P>0.05; The positive actual state is Positive.

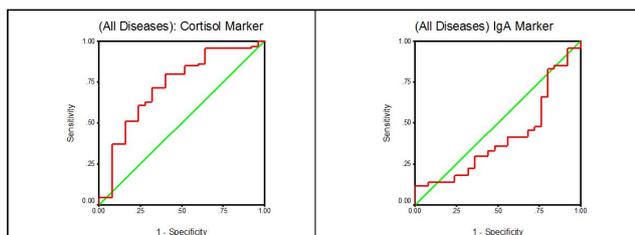


Figure 1: ROC curve plots of all disease's groups in admixture formed regarding studied markers.

DISCUSSION

Numerous studies on stress have pointed on several salivary stress biomarkers as indicators of stress reactions. So, salivary cortisol level is brought into relation by the hypothalamic pituitary-adrenal (HPA) axis activation [21]. In addition, because the immunosuppressive effect shown by stress, the level of secretory IgA [22] in saliva is used as a stress reactive biomarker. Estimation the levels of these salivary stress biomarkers (cortisol and IgA) in stressed individuals will aid to know that the stress as a one of the probable causes that explains the change in the levels of these salivary biomarkers. The salivary samples were collected from individuals; aged 15 years and over. The case group was stressed individuals with stress related oro-facial conditions and arranged into five subgroups (cheek biting, RAS, herpes labialis, tongue indentation, and TMJDs), their mean age was 23.94 years. The control group comprised of volunteers without stress, their mean age was 30.10 years. This study showed that there was a non-significant difference between the studied groups regarding gender. This result parallels other study done previously by Kania [23] who revealed that the difference in stress levels between males and females was insignificant.

Salivary cortisol is usually used as a biomarker of stress and related physical or mental diseases [15]. That agrees with the results of this study which show salivary cortisol is a useful biomarker for evaluating psychological stress. The results showed a high reading of salivary cortisol level was registered in comparison with that of the control group. There was a highly significant difference (Asymp. Sig.=0.001) between the study group and control group as shown in table II.

Cheek biting as a linea alba or traumatic ulcer may be Psychological related [24]. The results of this study

showed a highly significant difference (P= 0.000) was present between the case group of cheek biting and control group. The trimmed mean of salivary cortisol in control group was (83.17ng/ml) while in cheek biting group was (317.72 ng/ml).

Recurrent aphthous stomatitis (RAS) is a usual condition in which ovoid or round painful ulcers occur on the oral mucosa repeatedly. It is considered that psychological disturbances, for example stress and anxiety play an important role in the incidence of RAS [25]. In this study, the association between the salivary cortisol level and RAS was analyzed. The results showed a highly significant difference was accounted in at least at P<0.05 between control group and RAS group. This agrees with a previous study by Karthikeyan et al. [26] who found a high significant cortisol level in the RAS group in comparison with the control group and they suggested that a higher psychological stress level was present in the study group in comparison with the control group indicating that the psychological stress can play a role in the RAS manifestation.

Tongue indentation is a term for the appearance of tongue when indentations are present along the sides (the lateral borders), because of compression of the tongue opposing the adjacent teeth [27]. The results of this study showed (P=0.06) that can be reported as a significant difference between the case group of tongue indentation and the control group in correlation with the salivary cortisol level. As far as we know, there are no studies considering salivary cortisol level in stressed individuals to compare with the results of the current study.

Recurrent herpes labialis: a form of infection by herpes simplex virus (HSV-1). The reactivation occurs in the presence of aggravating factors and one of these factors is the emotional stress [28]. This result showed that there was a non-significant difference reported between the study group of recurrent herpes labialis and the control group according to the salivary cortisol level. This result disagrees with a previous study by Mehta et al [29] who confirmed that astronaut undertaking a long-duration spaceflight experience both increased latent viral reactivation and changes in diurnal trajectory of salivary cortisol concentrations. The statistical difference between this current study and the previous study may be attributed to a more stress level that the astronauts subjected in comparison with the participants of this study.

The factors that can prolong the symptoms of the temporo-mandibular joint disorders are stress and parafunction [30]. The results of this study showed that there was no significant difference reported between the study group of TMJ disorder and control group in relation with salivary cortisol level. This result is in agreement with Takatsuji et al [31] who observed no significant differences in the concentration of salivary cortisol before and after the student's examination, so these findings suggested that the acute stress because of the examination is not associated with salivary cortisol elevation. While this result disagrees with a previous result by Ali & Hadi [32] who studied salivary cortisol levels in 80 stressor students with a temporo-mandibular disorder and found significantly higher salivary cortisol levels than the controls. The cause of the non-significant difference of the result of this study may be due to the lower number of participants with TMD in comparison.

Considering IgA: There was a non-significant difference (Asy. Sig.= 0.179) in salivary IgA level between the general group of stress and control. This result agrees with a study by Lambert et al [33]. The authors indicated a normal SigA profile in stressed women who are breast cancer survivors in comparison with women without history of cancer. But, the result of the present study showed that there was a highly significant difference in salivary IgA level between study group of herpes labialis and control group and this is in agreement with a study by Shirtcliff et al [34]. The authors found higher significant HSV-sIgA levels in the physically abused and post institutionalized adolescents than in participants who had a normal rearing background.

In this study, the result showed that there was a non-significant difference in salivary IgA level between recurrent aphthous stomatitis group and control group and this disagrees with a previous study by Brozović et al [35] who noted that IgA values in saliva differed significantly in individuals with minor RAU (recurrent aphthous ulceration) during the acute stage in comparison with controls. The cause of the statistical difference with the present study may be because of the collection of all salivary samples was in the acute stage of RAS in the previous study in comparison.

The result of this study showed that there was a non-significant difference in salivary IgA level between study group of tongue indentation and control group. Also, the result of this study showed a non-significant difference in salivary IgA level between study subgroups of both of (cheek biting and tongue indentation) and control group. We did not find a study considering salivary IgA level in stressed patients with cheek biting or tongue indentation. The results of this study showed that there was a non-significant difference in salivary IgA level between TMD group and control group and this agrees with a study by Doepel et al [36] who found no significant changes when comparing salivary IgA levels before and after treatment TMD (temporo-mandibular disorder) patients.

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