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SALIVARY LEVELS OF α-AMYLASE & CORTISOL IN PATIENTS WITH RECURRENT APHTHOUS ULCERATION

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ABSTRACT

Objective: Psychological stress is one of the prime triggering factors for recurrent aphthous ulceration. The goal of this investigation is to measure salivary levels of α-Amylase and Cortisol, as stress biomarkers, in patients with recurrent aphthous ulceration.

Subjects and Methods: Whole unstimulated salivary samples were collected from 25 patients with recurrent aphthous ulceration & 25 healthy controls. Salivary levels of α-Amylase and Cortisol was quantified using enzyme-linked immunosorbent assay (ELISA).

Results: A statistically significant increase in salivary levels of both α-Amylase and Cortisol was detected in recurrent aphthous ulceration patients compared to healthy controls.

Conclusion: Salivary α-Amylase and Cortisol can be used as reliable stress biomarkers in patients with recurrent aphthous ulceration.

KEYWORDS: Recurrent aphthous ulceration, salivary α-amylase, salivary Cortisol, psychological stress.

INTRODUCTION

Recurrent aphthous ulceration (RAU) is one of the most common oral mucosal disorders characterized by recurrent, painful, solitary or multiple, self-limiting oral ulcerations affecting mainly the non-keratinized oral mucosa[1,2]. Clinically, RAU has three distinct types: minor, major & herpetiform with the minor type being the most common accounting for almost 85% of the cases[3,4]. Epidemiologically, RAU has a prevalence ranging from 10% to 25% among the general population[4,5]. Despite the increased clinical prevalence of RAU, its pathogenesis remains unclear[6]. Several diverse factors have been implicated in triggering the disease pathogenesis such as psychological stress, genetic background, immunological disturbance, nutritional deficiencies (vitamin B12, iron and folate) and microbial factors[6-8]. Psychological stress has been identified as a major precipitating factor for RAU[9,10].

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Physiologically, stress triggers the stimulation of two main systems: the sympathetic nervous system (SNS) & the hypothalamic-pituitary-adrenal (HPA) axis. Stimulation of the SNS will ultimately results in the release of salivary α-Amylase while activation of the HPA axis leads to secretion of Cortisol [11-13].

In the last few years, salivary α-amylase has received a great attention as a potential stress biomarker of the SNS [14]. Salivary α-Amylase is an enzyme secreted by acinar epithelial cells of salivary glands, mainly the parotid glands. It accounts about 10% to 20% of the total salivary constituents & it is primarily involved in the digestion of starch in the oral cavity to glucose and maltose [15]. Several studies have revealed increased levels of salivary α-amylase following either physical or psychological stresses [14,16,17]. Thus, salivary α-amylase is now recognized as a fast, reliable & non-invasive biomarker of stress & anxiety [12,18,19].

Cortisol is a well-known stress hormone released from the cortex of the adrenal gland & mediates several important functions such as regulation of carbohydrates, protein & fat metabolism as well as maintenance of vascular reactivity & regulation of blood cell numbers [20]. Increased levels of Cortisol have been reported in patients with burning mouth syndrome, atypical facial pain & in patients who had confronted stress during dental procedures [21]. Cortisol can be quantified in blood & saliva. However, measurement of Cortisol in saliva is preferred as collection of saliva is simple, safe, non-invasive and relatively stress-free to the patient avoiding a possible increase in Cortisol levels resulting from fear of venipuncture [22,23].

Although stress has been documented as a triggering factor for the development of RAU, the exact underlying pathomechanism remains unclear & controversial. Therefore, the goal of the current study is to quantify salivary levels of α-Amylase & Cortisol in patients with RAU during the active ulcerative stage.

PATIENTS AND METHODS

Study Population

A total of fifty subjects were included in this case-control study. They were all recruited from the outpatient clinic of Oral Medicine, Diagnosis and Periodontology Department, Faculty of Dentistry, Cairo University between September and December 2017. Participating subjects were equally divided into two, age and sex matched, groups; Group 1 or patients group consisted of twenty five patients with RAU (10 males and 15 females). Their ages range from 19 to 36 years (mean age: 26.12 years). Group 2 or Control group comprised twenty five systemically healthy subjects (9 males and 16 females). Their ages range from 18 to 37 years (mean age: 27.16 years).

Inclusion and exclusion criteria

Inclusion criteria consisted of systemically healthy patients presenting clinically with typical minor RAU during the active ulcerative stage with history of ulcer recurrence at least three times per year.

Exclusion criteria consisted of the presence of other diseases or conditions, such as anemia, cyclic neutropenia, Behcet's disease, inflammatory bowel disease and pregnancy. Smokers & patients who had received any medication that might influence their immune status or the salivary flow within the last three months were also excluded.

Full medical history was obtained from all participating subjects according to the detailed questionnaire of the modified Cornell Medical Index [24]. The Institutional Review Board approved the study protocol and all included subjects agreed to join this study and signed an informed written consent.

Salivary Samples collection

All participating subjects were requested to refrain eating & drinking at least two hours before

sample collection. All Salivary samples were obtained in the morning between 9:00 to 11:00 a.m. Before sample collection, subjects were asked to rinse their mouth using distilled water and after five minutes, whole unstimulated saliva samples were obtained using the simple standard technique by Navazesh [25]. Subjects were asked to sit comfortably and to expectorate into plastic tubes to obtain 5 ml of saliva. All salivary samples were then centrifuged at 4000xg for 10 minutes at 4 ºC, the upper parts were drawn and stored in small aliquots at - 20ºC until assayed.

Determination of Salivary Cortisol level

Salivary levels of Cortisol were determined in all collected samples using enzyme linked immunosorbent assay (ELISA) kit (Salimetrics, State College, PA, USA) following manufacturer's guidelines. This kit is a competitive immunoassay. Cortisol in samples and standards competed with Cortisol conjugated to horseradish peroxidase for the antibody binding sites on a microtitre plate. After incubation, unbound components were washed away and bound Cortisol enzyme conjugate was measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine. The optical density was read on a standard plate reader at 450 nm.

Determination of Salivary α-amylase level

Salivary levels of α-amylase were determined in all obtained salivary samples using enzyme linked immunosorbent assay (ELISA) kit (Salimetrics, State College, PA, USA) following manufacturer's instructions. This kit uses the chromogenic substrate 2-chloro-p-nitrophenol linked with maltotriose. The enzymatic action of α-Amylase on this substrate resulted in 2-chloro-p-nitrophenol which was spectrophotometrically measured at 405 nm.

Statistical analysis

All obtained data are presented as mean and standard deviation (SD) values (mean ± SD). Unpaired Student's t-test was used to compare between α-Amylase & Cortisol levels in saliva of RAU patients and healthy control group. Statistical tests were carried out by the GraphPad statistical software (Graph Pad Software Inc, La Jolla, CA). P value is statistically significant at < 0.05.

RESULTS

The present study comprised a total of fifty subjects divided into two equal groups; RAU group & healthy control group. Demographic data of the two studied groups is presented in Table 1. Table 2 shows the mean salivary levels of Cortisol & α-Amylase in patients with RAU and healthy controls (figure 1 & figure 2). Results of our study revealed a statistically significant increased levels of both α-Amylase & Cortisol in saliva of RAU patients compared to healthy control group ($p<0.05$).

TABLE (1) Demographic data of the two studied groups

Study Groups	Number	Age (years) (mean ± SD)	Sex (M/F)
RAU group	25	26.12 ± 5.54	10/15
Control group	25	27.16 ± 5.49	9/16

TABLE (2) Salivary α-Amylase & Cortisol levels in both studied groups

Salivary biomarker	RAU Group (n=25)	Control Group (n=25)	P value
α-Amylase (U/mL) (mean ± SD)	192.37±101.35	128.27±82.94	0.0181*
Cortisol (µg/dL) (mean ± SD)	1.53 ± 0.69	1.02 ± 0.48	0.0039*

Statistically significant difference (Unpaired Student t-test; p<0.05)

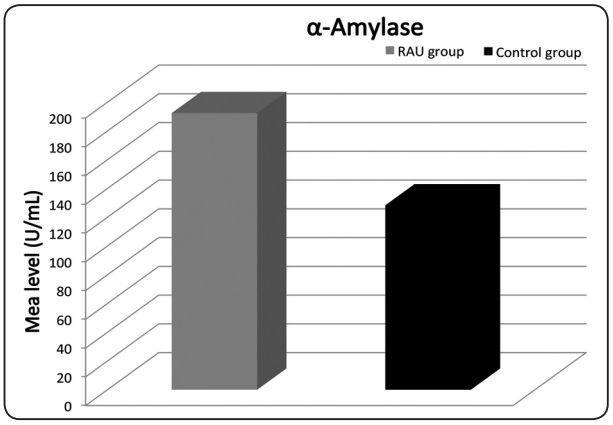

Fig. (1) Bar chart representing the mean levels of salivary α-Amylase among RAU group and Control group

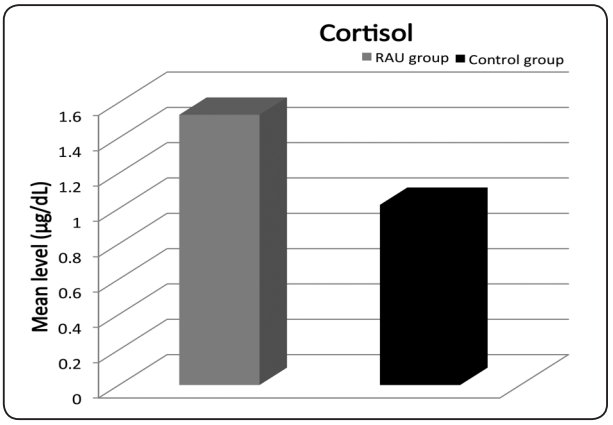

Fig. (2) Bar chart representing the mean levels of salivary Cortisol among RAU group and Control group

DISCUSSION

Several previous studies have proposed that psychological stress may contribute to the onset & recurrence of RAU. However, results of these studies were heterogeneous[26-31]. Salivary biomarkers such as α-Amylase & Cortisol have been proved to play a crucial role in human responses to psychological stressful circumstances[32]. Hence, the aim of this study was to evaluate the salivary levels of α-Amylase & Cortisol, as stress biomarkers, in patients with RAU during the active ulcerative stage, in a trial to illustrate their possible role in RAU etiopathogenesis. The current study is the first to evaluate salivary levels of both biomarkers in patients with RAU. We investigated the salivary levels of these two biomarkers in twenty-five patients with RAU and compared it to twenty-five healthy control subjects. Our results revealed statistically increased levels of both, α-Amylase & Cortisol, in saliva of RAU compared to healthy controls. These results suggest that salivary α-Amylase & Cortisol can be used as promising useful salivary biomarkers in RAU and further support the role of stress in triggering the pathophysiology of RAU.

Stress is a multidirectional process that can be assessed through psychological questionnaires &/or biological markers. Biological stress markers are preferred over stress & anxiety questionnaires as they present an objective, reliable & genuine proof of stress that is less vulnerable to exaggeration[14, 33]. Indeed, Neuro-endocrine markers play a major role in human reactions to stressful situations [34]. Specifically, salivary α-Amylase & Cortisol, as previously mentioned, are authentic biological stress markers released under the regulation of the SNS & the HPA axis, respectively[35]. In the last ten years, salivary α-Amylase has gained a great attention as a stress biomarker [12] whereas salivary cortisol has been a noteworthy & reliable stress biomarker since nearly twenty years [36].

Cortisol is a pivotal hormone in reaction to stress. *Elenkov*[37] has reported that stress leads to increase in Cortisol levels which affect the immune system response & ultimately results in imbalance in Th1/Th2 cytokines. Meanwhile, *Borra et al*. [38] have revealed that RAU formation is associated with an imbalance of these cytokines, with increased Th1 activity. Interestingly, results of the current study showed statistically significant increase in salivary levels of Cortisol among RAU patients compared to healthy controls. Our results are partially in accordance with those of *McCartan et al*[27]**,** *Albanidou-Farnaki et al*[30]*, Nadendla et al*.[39]*, Karthikeyan & Aswath* [10] and *Vassandacoumara & Daniel* [40] who reported increased salivary Cortisol levels in patients with RAU. On the other hand &

partly in contrast to our results, *Michel et al*.[41], *Rezaei et al*.[42] & *Kunikullaya et al*.[43] reported no significant difference between salivary levels of Cortisol in RAU patients compared to controls. However, they demonstrated an association between RAU with stress & anxiety. The discrepancy observed in results of these studies may be due to different ethnic & environmental factors together with small sample sizes.

The increase in salivary Cortisol levels observed in our results among RAU patients can be explained on the basis that stress stimulates the HPA axis leading to secretion of corticotrophin-releasing hormone (CRH) from the hypothalamus. CRH then induces the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. Finally, ACTH induces the release of Cortisol from the adrenal cortex[44]. Increased Cortisol levels will then lead to various immune & inflammatory responses eventually resulting in imbalance in Th1/Th2 cytokines production which is implicated in the pathogenesis of RAU[37,38,44,45]. Moreover, *Bellavance & Rivest*[46] had reported that despite the well-recognized anti-inflammatory properties of glucocorticoids there exists increasing evidence indicating that glucocorticoids stimulate inflammatory responses under specific conditions. Interestingly, the great majority of pro-inflammatory actions of glucocorticoids were seen in association with stress[46-48].

Salivary α-Amylase has been investigated in several studies as a valid & reliable stress biomarker of the SNS [12, 49, 50]. *Rashkova et al*. [50] have reported a two-fold increase in salivary levels of α-Amylase in stressful situations compared to its levels in stress free situations. In this study, results have shown that, compared to healthy controls, salivary α-Amylase levels were statistically significantly increased among RAU patients. Consistent with our results, *Vineetha et al*. [51] reported increased salivary levels of α-Amylase in patients with chronic psychosocial stress mainly in RAU & patients with dry mouth. They concluded that salivary α-Amylase can be used as a chronic stress biomarker [51]. Furthermore, *Kunikullaya et al*.[43] reported a mild increase in salivary α-Amylase among patients with RAU but this increase was not statistically significant from the healthy controls. In contrary to our results, *Cardoso et al*. [52] revealed no significant difference between salivary levels of α-Amylase in RAU patients when compared to healthy controls.

Increased levels of salivary α-Amylase observed in this study may be explained on the basis that stress induces the SNS to produce salivary α-Amylase from salivary glands through the secretion of catecholamines, mainly norepinephrine[53]. Indeed, stress leads to disturbance in the psycho-neuro-immune balance & enhances stress-induced cytokine production that may contribute to the pathophysiological process of RAU[44].

In conclusion, results of this study showed that salivary α-Amylase and Cortisol levels are increased among RAU patients. Both, salivary α-Amylase and Cortisol can be used as reliable stress biomarkers in patients with RAU.

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