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Analysis of Salivary Cortisol and Amylase Levels in Patients with Oral Ulcers, A Case- Control Study

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ABSTRACT

Background: Oral ulcers are common inflammatory lesions of the oral mucosa caused by factors including local trauma, systemic disease, and psychosocial stress. The changes in the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system under stress can be measured non-invasively by salivary cortisol and α-amylase levels. The purpose of this study was to determine the salivary cortisol and α-amylase in patients with oral ulcers compared to healthy controls, and to assess their associated clinical factors.

Methods: A case-control study was conducted among 18-60 years old, 126 participants (63 patients and 63 controls). The unstimulated whole saliva samples were collected between 8:00-10:00 a.m. under standardized pre-sampling conditions. Cortisol levels were measured via Enzyme-linked Immunosorbent Assay (ELISA), and α-amylase activity was quantified through a kinetic colorimetric technique. SPSS v26 was used for data analysis. Independent t-test and the chi-square test were used for variables measurement. $p < 0.05$ was considered significant.

Results: Salivary cortisol levels were significantly higher in cases (8.42 ±1.25) ng/ml compared to controls (6.23 ± 1.12; $p < 0.001$). Mean salivary α-amylase was also increased in cases (162.5 ± 28.7 U/mL) than in controls (129.8 ± 25.4 U/mL; $p < 0.001$). Recurrent lesions (45 (71.4%)), insufficient sleep (38 (60.3%)), and recent psychological stress (42 (66.7%)) were more commonly reported in ulcer patients.

Conclusion: High levels of salivary cortisol and α-amylase in oral ulcer patients indicate neuroendocrine involvement in ulcer development as a stress reaction. These biomarkers can be valuable tools in diagnosis, prognosis, and stress-focused management.
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Keywords: Oral Ulcer, Stomatitis, Amylases, Sympathetic Nervous System

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INTRODUCTION

Among the most common types of oral mucosal lesions are oral ulcers, particularly recurrent aphthous stomatitis (RAS), which has an estimated prevalence of 5-25 % of the global population [1]. These lesions are often associated with improper nutrition and diminished quality of life. Local trauma, systemic diseases, nutritional deficiency, and psychosocial stress are factors involved in their development [2]. Stress activates the sympathetic adrenal-medullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis, resulting in measurable changes in salivary cortisol and alpha-amylase levels [3]. Salivary cortisol reflects HPA axis activity, while α-amylase serves as an indirect marker of the sympathetic nervous system, and both are reliable and non-invasive indicators of physiological stress [4].

Increased salivary cortisol or α-amylase levels are present in multiple stress-related oral diseases, whereas decreased or non-altered levels can be found in individuals with no active lesions [5]. These disparities suggest a biological association between neuroendocrine stimulation and oral ulcers [6].

Nevertheless, the results are inconsistent due to differences in sampling time, assay methodology, and population characteristics [7]. Moreover, there are limited studies that assessed both biomarkers simultaneously in clearly characterized populations with standardized procedures [8]. Recognizing this relationship may enhance awareness of people at higher risk of stress-induced oral ulcers and guide any preventive or treatment measures [9]. Profiling biomarkers may also facilitate the introduction of stress analysis into the standard oral healthcare framework, decreasing instances of recurrence and enhancing patient outcomes [10].

This study aimed to compare early-morning salivary cortisol and α-amylase in patients with oral ulcers and age and sex-matched healthy controls. The purpose was also to determine their associated clinical features, including history of recurrent ulcers, sleep quality, and recent psychological stress

METHODS

A cross-sectional comparative study compared salivary cortisol and α-amylase levels between patients with oral ulcers and healthy individuals, conducted at the Dental College Superior University and SZH Lahore From February to June 2025 (Ref: 4426/25), after an informed consent. A Consecutive non-probability sampling technique was used to recruit participants. Cases were selected at the time of clinical diagnosis of oral ulcers, and controls were selected from healthy attendants or hospital visitors without oral lesions. Informed written consent was obtained from all the participants. The sample size of 126 participants (63 cases and 63 controls) was calculated using OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) based on $\alpha = 0.05$, power = 80%, and an anticipated moderate effect size ($d = 0.5$) [11].

The inclusion criteria for participants were 18-60 years old, willing to provide saliva samples, and having no systemic illnesses affecting salivary biomarkers. The exclusion criteria were pregnancy, lactation, smoking, alcohol consumption, acute infection, taking corticosteroid or β-blocker, and independent refusal. Instead of active intervention, the study focused on natural exposures (presence or absence of oral ulcers). The variables of study included socio-demographic (age, sex, BMI), clinical (ulcer recurrence, sleep quality, recent psychological stress, caffeine intake), and biochemical variables (salivary cortisol and α-amylase).

The saliva samples were collected between 8:00 and 10:00 a.m., after at least one hour without food, drink, or oral hygiene. The enzyme-linked immunosorbent assay (ELISA) measured salivary cortisol, and a kinetic colorimetric assay was employed to measure α-amylase activity. SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY) was used for data analysis. Chi-square tests were used to compare categorical variables, and independent t-tests were used for continuous variables. The statistical significance was considered at p-value < 0.05.

RESULTS

Table 1: Demographic Characteristics of Study Participants

Variable	Category	Cases (n=63)	Controls (n=63)	Test Value	p-value
Age (years)	Mean ± SD	34.8 ± 9.6	33.9 ± 8.7	t = 0.53	0.598
Sex	Male	28 (44.4%)	27 (42.9%)	$\chi^2 = 0.03$	0.863
BMI (kg/m²)	Mean ± SD	24.7 ± 3.2	24.4 ± 3.0	t = 0.50	0.616
Smoking status	Yes	12 (19.0%)	10 (15.9%)	$\chi^2 = 0.20$	0.655
	No	51 (81.0%)	53 (84.1%)		

*n = Number of participants, BMI = Basal Metabolic Index, SD = Standard Deviation, % = Percentage, * = Significance at p<0.05*

This study assessed salivary cortisol and α-amylase as stress biomarkers in 126 individuals (63 = oral ulcer patients and 63 = controls). The demographic variables were similar across groups. Recurrent lesions, sleep disturbance, and recent stress were more commonly reported among ulcer patients. There was a significant increase in both biomarkers in cases ($p < 0.001$). The findings support the use of salivary biomarkers to detect stress-induced mouth ulcers and their management. Demographic characteristics of study participants are indicated in **Table 1.**

Table 2: Clinical and Potential Confounding Factors

Variable	Category	Cases (n=63)	Controls (n=63)	Test Value	p-value
History of recurrent ulcers	Yes	45 (71.4%)	0 (0.0%)	$\chi^2 = 75.88$	<0.001*
Systemic disease	Present	8 (12.7%)	5 (7.9%)	$\chi^2 = 0.74$	0.390
Sleep quality	Poor	38 (60.3%)	19 (30.2%)	$\chi^2 = 11.33$	0.001*
Recent psychological stress	Yes	42 (66.7%)	21 (33.3%)	$\chi^2 = 14.88$	<0.001*
Caffeine intake	≥2 cups/day	29 (46.0%)	24 (38.1%)	$\chi^2 = 0.84$	0.360

*n = Number of participants, % = Percentage, * = Significance at p<0.05*

The mean age was similar between cases (34.8 ± 9.6 years) and controls (33.9 ± 8.7 years, p = 0.598), with equal sex distribution and BMI. This similarity suggests that the two groups were demographically equivalent, thus minimizing baseline confounding by matching biomarkers. Clinical and potential confounding factors associated with oral ulcers are presented in **Table 2.**

Table 3: Salivary Biomarker Levels in Cases and Controls

Biomarker	Group	Mean ± SD	Test Value	p-value
Salivary Cortisol (ng/mL)	Cases	8.42 ± 1.25	t = 8.74	<0.001*
	Controls	6.23 ± 1.12		
Salivary α-Amylase (U/mL)	Cases	162.5 ± 28.7	t = 7.19	<0.001*
	Controls	129.8 ± 25.4		

*n = Number of participants, SD = Standard Deviation, * = Significance at p<0.05*

Prevalence of recurrent lesion (45 (71.4%) vs. 0 (0%); p < 0.001), poor sleep quality (38 (60.3%) vs. 19 (30.2%); p = 0.001), and recent occurrence of psychological stress (42 (66.7%) vs. 21 (33.3%); p < 0.001) were significantly higher among ulcer patients. These results support the role of psychological and lifestyle factors in the incidence of ulcers. Table 3 illustrates the levels of salivary biomarkers in cases comparable to controls.

Mean salivary cortisol was significantly higher in cases (8.42 ± 1.25 ng/mL) compared to controls (6.23 ± 1.12 ng/mL; $p < 0.001$). Cases demonstrated significantly higher salivary α-amylase levels (162.5 ± 28.7 U/mL vs. 129.8 ± 25.4 U/mL; $p < 0.001$). These findings suggest stress-related neuroendocrine activation in patients with oral ulcers.

DISCUSSION

The purpose of this study was to compare salivary cortisol and α-amylase in patients with oral ulcers and healthy controls, and to assess their relationships with ulcer recurrence history, sleep quality, and psychological stress. These findings support that patients with oral ulcers demonstrate significantly elevated levels of salivary cortisol and α-amylase compared to healthy subjects, suggesting an increased neuroendocrine response involving stress.

There were no significant demographic effects in cases in terms of age, sex, or BMI. Similar demographic matching has been observed in studies of oral mucosal disease, which reduced confounding in biomarker interpretation [12]. Clinically, oral ulcer patients experienced recurrent lesions, low sleep quality, and recent psychological stress more frequently. These aspects have also been associated with increased risk and severity of RAS in similar studies [13]. Similarly, studies have linked inadequate sleep to high blood cortisol and sympathetic activity, which may facilitate the recurrence of lesions [14].

The ulcer group had a significantly higher level of salivary cortisol. Consistent with these results, active RAS elevations relative to remission or controls have been reported [15]. Other studies have also linked cortisol excess to poor mucosal repair [16]. Patients with oral ulcers also demonstrated significantly increased salivary α-amylase levels. Similar elevations have been reported in inflammatory oral diseases associated with increased sympathetic drive [17]. Simultaneous increases in cortisol and α-amylase have been reported in oral diseases related to psychosomatic factors [18].

These results align with the literature, indicating that both biomarkers are indices of integrated HPA axis and sympathetic activation in stress-related oral disorders [19]. Other studies did not observe differences in clinically significant biomarkers, indicating differences in timing of collection or lesion stage [20]. These discrepancies may be attributed to pre-analytical factors, including pre-sampling dietary intake or stimulant use [21]. Evidence that stress mediators regulate inflammatory cytokines and slow healing is biologically plausible [22]. Biomarker detection advances enhance precision in measures, whereas standardized protocols are assumed to increase reproducibility [23,24]. The use of these biomarkers in oral care may allow early detection of high-risk people and enable specific interventions, including stress reduction or sleep improvements [25,26].

The limitations include the single-center setting, the case-control study design that limits causal inference, the use of a single time-point biomarker without considering diurnal variation, and potential residual confounding by unmeasured factors such as diet, oral hygiene, or systemic illness. Future research should consider multicenter longitudinal designs, repetitive sampling, and assess targeted stress-reduction interventions for ulcer prevention.

CONCLUSION

In this study, elevated salivary cortisol and α-amylase were substantial and positively correlated among patients with oral ulcers, providing evidence of stress-related neuroendocrine activation. These results support the association of oral ulcers with elevated biomarkers. This is further corroborated by psychological stress, poor sleep, and patterns of recurrence.

These findings highlight the importance of salivary cortisol and α-amylase as non-invasive biomarkers in diagnosis, prognosis, and stress-directed treatment. Biomarker testing combined with psychosocial assessment may help lower recurrence and improve oral healthcare outcomes.

LIST OF ABBREVIATIONS

HPA	Hypothalamic-Pituitary-Adrenal (Axis)
SAM	Sympathetic-Adrenal-Medullary (System)
RAS	Recurrent Aphthous Stomatitis
ELISA	Enzyme-Linked Immunosorbent Assay
BMI	Basal Metabolic Index

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

A cross-sectional comparative study compared salivary cortisol and α-amylase levels between patients with oral ulcers and healthy individuals, conducted at the Dental College Superior University and SZH Lahore From February to June 2025 (Ref: 4426/25), after an informed consent.

AUTHORS' CONTRIBUTION

All authors contributed equally as per ICMJE policy

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