

*Case Report*

# Comparative Assessment of Salivary Level of Cortisol, Anxiety and Depression in Patients with Oral Conditions: A Case Control Study

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## Abstract

**Introduction:** Cortisol, as the main human glucocorticoid, is considered to be a biological marker of stress and anxiety. It is suggested that stress with its presumed effects on the immune system, constitutes one of the major causative agents for oral mucosal alterations.

**Methodology:** To enable the study of gender-based differences in stress, salivary alpha-amylase levels and oral lesions, an equal gender distribution was guaranteed. The individuals were then given a clinical examination to look for oral mucosal alterations caused by stress, such as recurrent aphthous ulcers, oral lichen planus, dry mouth and burning mouth syndrome. To identify Temporomandibular Joint Disorders (TMDs), which are also linked to stress, trigger points, masticatory muscles and TMJs were examined.

**Results:** 80 participants made up the research team, ranging in age from 24-47 years for the experimental group and 27-50 years for the control group. There was an equal number of men and women in both groups. The investigation group's average age was 33.70 years, compared to 31.93 years for the control group ( $p=0.16$ ). The study group's female participants exhibited stress levels that ranged from 5% ( $N=1$ ) for light stress to 40% ( $N=8$ ) for severe stress ( $p=0.10$ ). **Conclusion:** However, there was no connection between oral alterations and salivary alpha-amylase.

**Keywords:** Salivary Alpha-Amylase; Oral Changes; Oral Lichen Planus; Cortisol

## Introduction

Cortisol, as the main human glucocorticoid, is considered to be a biological marker of stress and anxiety. It is suggested that stress with its presumed effects on the immune system, constitutes one of the major causative agents. Previous studies report increased frequency of oral ulcer manifestation in intensely stressed populations. Thus, the psychological-emotional factors seem to be major predisposing factors for oral mucosal alterations. An insight into a patient's psychological status can be estimated from both serum free and salivary cortisol levels. Cortisol, also called as a stress hormone, has been used as an indicator in the stress evaluation studies. The assessment of cortisol in saliva has gained interest in studies for evaluating anxiety. Salivary cortisol may actually provide a better measure than serum cortisol of the stress response as it more accurately measures the amount of unbound cortisol compared to serum measures. Salivary cortisol exhibits a clear diurnal variation and circadian rhythmicity with a time course closely parallel to that of plasma cortisol [1].

Although the cause is unknown, various theories involving genetic, viral, psychogenic and autoimmune aspects exist. This disease believed to be caused by a cell-mediated immune response, in which Langerhans cells, keratinocytes and activated T-lymphocytes are implicated, according to significant research on the pathophysiology [2].

In the modern world, stress has become a constant part of our hectic lives. Stress is characterized as the mental and physical state that develops when an individual's capabilities are insufficient to meet the demands and pressures of a certain circumstance [3,4]. Adverse health outcomes like heart disease, cancer, gastrointestinal problems, migraines and oral ailments have all been linked to psychological stress [5].

Questionnaires can be used to subjectively analyze stress and different biomarkers can be used to quantitatively assess stress. In the medical field, a number of questionnaires are employed, including the Hospital Anxiety and Depression Scale (HAD), State-Trait Anxiety Inventory, Perceived Stress Scale and Depression Anxiety and Stress Scale. The HAD scale is a widely used, straightforward and user-friendly instrument for measuring anxiety and depression. It has already been used in numerous research and is helpful for the first identification of psychological illnesses [6]. The best and least invasive biological source for measuring stress mediators on a quantitative and qualitative level is saliva. Chromogranin A, salivary alpha-amylase, salivary IgA, cortisol and lysozymes are a few of the several salivary biomarkers [7].

Cortisol has been extensively researched and is now considered the "gold standard" biomarker in the study of stress for assessing HPA axis dysfunction. Salivary alpha-amylase is currently gaining recognition as a reliable and valid indicator of ANS activation. Collectively, these systems interact with the immune system and play a significant role in the development of disorders linked to stress [8]. Salivary Alpha-Amylase (sAA) is thus useful as a biomarker to assess the psychological health of those who work in demanding environments [9].

The initial goal of this research was to measure the stress levels and salivary levels of alpha-amylase in corporate personnel. The investigation also sought to determine the incidence of oral conditions caused by stress and any connections between salivary amylase levels, stress levels and related mouth conditions.

## Methodology

Eighty patients who visited the outpatient department of the Oral Medicine and Radiology, Dental College, in Azamgarh, Uttar Pradesh, were included in this study. They age from 25 to 60 years, were employed in the corporate sector as software engineers or higher cadres and also had at least three years of experience working for the same company were included in the study. At the same time, expectant or nursing mothers, people with hypertension, anemia, kidney or endocrine disorders, those taking oral contraceptives or corticosteroids, smokers and people with diseases of the salivary glands were excluded. Before involving subjects in the study, the protocol was thoroughly explained to them and their written informed consent was obtained. Prior to starting the study, the Institutional Committee and Review Board of Dental College provided their ethical approval. The Helsinki Declaration from 1975 guided the research's methodology.

For the purpose of the research, a total of 80 participants were chosen. On the basis of the study's power and the confidence interval of 90% ( $p < 0.05$ ), the sample size was chosen. The Hospital Anxiety and Depression scale (HADs) questionnaire, which was utilized as a screening tool, was used to examine the individuals' subjective assessment of their stress levels. Scores between 0 and 7 on the questionnaire are regarded as normal, between 8 and 10 as borderline and above 11 as "high stress." The participants were separated into the following groups based on their scores.

**Study Group (with stress):** Consisted of a total of 40 subjects comprising 20 men and 20 women with HADs score of 8 or more.

**Control Group (non-stress):** Consisted of a total of 40 subjects comprising 20 men and 20 women with HADs score of 7 or less.

To enable the examination of gender-based variations in stress, salivary alpha-amylase levels and oral lesions, an equal gender distribution was guaranteed. The patient's demographic information, medical and dental histories were all included in a detailed case history that was written down. The individuals were then given a clinical examination to look for oral mucosal alterations caused by stress, such as recurrent aphthous ulcers, oral lichen planus, dry mouth and burning mouth syndrome. To identify Temporomandibular Joint Disorders (TMDs), which are also linked to stress, trigger points, masticatory muscles and TMJs were examined. The proforma included documentation of every finding. The Spit Method was used for gathering unstimulated salivary samples (1 ml) in sterile saliva collection tubes. Patients were advised to refrain from intake of any food or beverages at least one hour before the collection of saliva. To prevent diurnal variations in salivary alpha-amylase, all salivary samples were taken from the patients between 10 and 11 AM. The samples were kept at - 20 degrees and later used to estimate the amounts of

salivary alpha-amylase using an enzyme-linked immunosorbent assay (Human Amylase 1 ELISA Kit). Following the determination of amylase concentrations, the statistical relationship between salivary alpha-amylase concentrations and stress levels as well as the prevalence of oral diseases in the two groups was examined.

The gender-based comparison of stress levels and the prevalence of related oral problems within study groups and between genders in the study groups was done using a test called the Chi-Square. The mean salivary amylase levels (ng/ml) were compared between the Study and Control groups, as well as between the genders within each group, using the Mann Whitney test. In the control group, similar comparisons were made between mean salivary amylase levels (in ng/ml) based on various stress levels across genders and, lastly, between various related dental illnesses. The mean Salivary Amylase levels (in ng/ml) based on various Stress levels in the Study group and between various related oral problems in the Study group were compared using the Kruskal Wallis Test followed by the Mann Whitney post hoc test.

## Results

The study consisted of 80 subjects with an age range of 24-47 years in the study group and 27-50 years in the control group. Both the groups had an equal distribution of males and females (Table 1). The mean age of subjects in the study group was 33.70 years and in the control group subjects, it was 31.93 years ( $p=0.16$ ). Among the males in the study group, it was noted that 30% ( $N=6$ ) had mild stress levels, 45% ( $N=9$ ) had moderate stress levels and 25% ( $N=5$ ) had severe stress levels. Salivary amylase levels were higher in the subjects with severe stress than they were in the subjects with mild and moderate stress ( $p=0.001$ ). The prevalence of concomitant oral problems was compared between the Study and Control groups and it was shown that in the former, 17.5% ( $n=7$ ) of patients presented with RAS, 7.5% ( $n=3$ ) with Dry mouth, 5% ( $n=2$ ) with TMDs and 2.5% ( $n=1$ ) with Dry mouth and TMD, while in the latter, 5% of patients presented with RAS alone. These variations were statistically noteworthy (Table 2). The linked disorders affected women more often than men. There wasn't no statistically significant difference in the mean salivary amylase levels between the Study and Control groups based on the related oral ailments, showing that there was no direct correlation between the amylase levels and the oral disorders (Fig. 1, Table 3).

| Variable | Category    | Study group |      | Control group |      | P-Value           |
|----------|-------------|-------------|------|---------------|------|-------------------|
|          |             | Mean        | SD   | Mean          | SD   |                   |
| Age      | Mean and SD | 33.7        | 5.88 | 31.93         | 5.16 | 0.16 <sup>a</sup> |
|          | Range       | 27 - 47     |      | 27 - 50       |      |                   |
|          |             | N           | %    | N             | %    |                   |
| Sex      | Males       | 20          | 50%  | 20            | 50%  | 1.00 <sup>b</sup> |
|          | Females     | 20          | 50%  | 20            | 50%  |                   |

**Table 1:** Age and gender distribution among two groups.

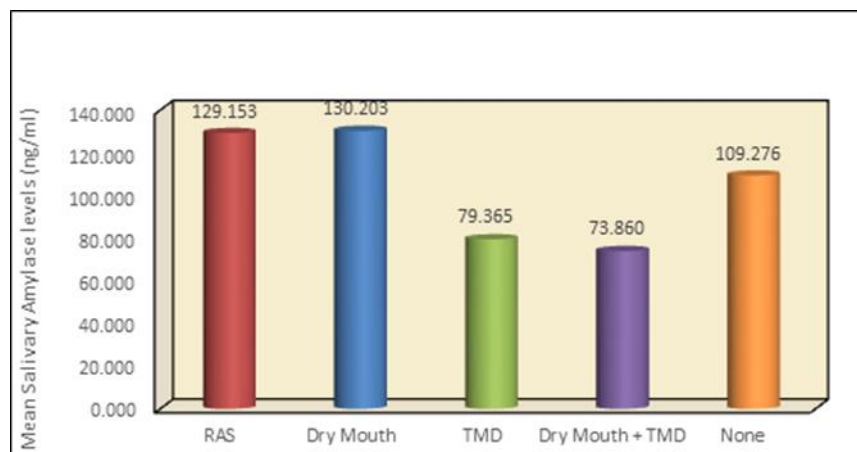
| Chi Square Test     |             |        |               |        |                |         |
|---------------------|-------------|--------|---------------|--------|----------------|---------|
| Associated disorder | Study group |        | Control group |        | $\chi^2$ value | P-Value |
|                     | N           | %      | n             | %      |                |         |
| RAS                 | 7           | 17.50% | 2             | 5.00%  | 10.639         | 0.03*   |
| Dry Mouth           | 3           | 7.50%  | 0             | 0.00%  |                |         |
| TMD                 | 2           | 5.00%  | 0             | 0.00%  |                |         |
| Dry Mouth + TMD     | 1           | 2.50%  | 0             | 0.00%  |                |         |
| None                | 27          | 67.50% | 38            | 95.00% |                |         |

**Table 2:** Comparison of prevalence of associated oral disorder between study and control groups.

| Group | Disorders | N | Mean | SD | Min | Max | P-Value |
|-------|-----------|---|------|----|-----|-----|---------|
|-------|-----------|---|------|----|-----|-----|---------|

|       |                 |    |         |        |       |        |      |
|-------|-----------------|----|---------|--------|-------|--------|------|
| Study | RAS             | 7  | 129.153 | 56.315 | 82.34 | 235.67 | 0.25 |
|       | Dry Mouth       | 3  | 130.203 | 53.195 | 95.03 | 191.4  |      |
|       | TMD             | 2  | 79.365  | 7.46   | 74.09 | 84.64  |      |
|       | Dry Mouth + TMD | 1  | 73.86   | .      | 73.86 | 73.86  |      |
|       | None            | 27 | 109.276 | 59.439 | 53.42 | 247.76 |      |

**Table 3:** Comparison of mean salivary amylase levels based on associated oral disorders in Study group using Kruskal Wallis test.



**Figure 1:** Mean salivary amylase levels (in ng/ml) based on associated oral disorders in study group.

## Discussion

There will always be psychological stress throughout life. The World Health Organisation predicted in 1996 that stress-related mental diseases will be quite common by 2020 [10]. It has been hypothesized that chronic stress is a significant contributor to changes in neuroendocrine, cardiovascular, autonomic and immune functioning, which result in a variety of disorders, some of which have oral manifestations connected to stress [4]. Stress has been a threat to people in practically all occupations' mental and physical wellbeing. The increased prevalence of negative impacts of the profession on employees' psychological and physical health is primarily driving interest in professional stress studies. In comparison to participants who were not professionally worried, those who were professionally stressed had a 10 times higher risk of acquiring psychiatric disorders and health problems, according to a study on stress among Indian software professionals [4,6,11]. Therefore, psychological stress, which is modern and unavoidable in the corporate sector, needs to be addressed and research involving this group at all levels needs to be encouraged. The HAD Scale was used in this study to evaluate the patients' psychological health. The scale was developed by Zigmond Snaith to assess patients' levels of anxiety and depression and it is now a popular tool in both clinical use and research. The validity of the HADS in the evaluation of anxiety and depression has been demonstrated in numerous investigations [6].

According to a 2018 study by Ajmal Muhammed, the HAD Scale was effective in determining the presence and severity of anxiety disorders and depression in both patients and the general population [12]. The participants were subsequently divided into groups with mild, moderate and severe stress levels based on the results of the HAD Scale. The amount of stress in women was found to be higher than in men. This may be explained by the fact that most females are the primary carers, must multitask and handle their personal and professional responsibilities. Following a study by Rani and Bhuvaneshwari K, it was discovered that working women between the ages of 29 and 32 experienced high levels of stress [13]. Within the research group, the participants who had high levels of stress also had statistically significant amounts of amylase. This indicates a beneficial relationship between salivary amylase and stress levels, pointing to the potential use of salivary amylase as a stress biomarker. A pharmacological investigation by Stegeren AV shows that salivary amylase is sensitive to variations in adrenergic activity, particularly in response to psychological stress [14,15]. Salivary amylase levels and stress levels were found to be significantly correlated by Rashkova Maya, et al., Amylase levels in the study group were statistically significantly higher in females than in

males in this current investigation [9].

This finding is consistent with research by Carr AR, et al., which demonstrated that women responded to stressors more quickly than men and showed a significant rise in salivary amylase from pre- to post-stress [16]. Oral disorders can develop as an outcome of emotional expression or as an indirect effect of psychological changes [17]. RAS, OLP, BMS, Dry mouth, Atypical facial discomfort, TMDs and other illnesses are some of the many conditions linked to stress. In this research, it was shown that there were more cases of stress-related oral diseases in the study group compared to the control group and that RAS (17.5%), dry mouth (7.5%) and TMDs (5%) were the most common disorders, all of which were statistically significant ( $p=0.03$ ). Numerous studies demonstrate a connection between psychological issues and changes in oral mucosa [8,12]. The prevalence of oral disorders did not significantly differ across genders. The test findings revealed that there was no statistically significant difference in the mean salivary amylase levels between the Study group and Control group based on the linked oral disorders, indicating that there was no definitive link between salivary amylase levels and oral disorders. According to a study by Vineetha R, et al., there is no connection amongst the occurrence of oral lesions and amylase [4].

## Conclusion

Within the constraints of this research, it can be hypothesized that participants in the research group experienced higher levels of stress, which were consequently associated with higher levels of salivary alpha-amylase and that there was a positive correlation between stress levels and oral changes. There was no link between salivary alpha-amylase and related oral alterations, though.

## Conflict of Interest

The authors have no conflict of interest to declare.

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