Assessment of Stress Levels and Oral Mucosal Changes Among Corporate Employees - An Observational Study

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Abstract

Background
Psychological stress has been implicated in various oral diseases like Recurrent aphthous stomatitis (RAS), Oral lichen planus (OLP), Burning mouth syndrome (BMS), Dry mouth, and Temporomandibular joint disorders (TMDs). People working in the corporate sector are particularly at high risk of developing stress and its associated disorders. Evaluation of psychological stress can be done by salivary alpha-amylase which has been considered as a biomarker of stress.

Methods
The study included 80 subjects within the age group of 25-60 years working in corporate sector, who were assessed for their stress levels using HAD Scale and accordingly categorized into a STUDY group and a CONTROL group. The STUDY group was composed of 40 subjects with HAD scores of more than 8 and the CONTROL group was composed of 40 subjects with HAD scores of 0-7 ensuring an equal distribution of genders in both groups. The subjects underwent a complete clinical examination to identify stress-related oral diseases. One ml of unstimulated saliva was collected from each subject that was quantified for salivary alpha-amylase. All the findings were subjected to statistical analyses.
Result
Statistically significant higher levels of salivary amylase levels were recorded in the study group. The salivary amylase levels were higher in females than in males. There was a positive correlation between stress levels and oral diseases. The commonly encountered stress-associated oral diseases in the study subjects were RAS, Dry mouth, and TMDs.

Conclusion
This study reiterates the correlation of salivary alpha-amylase and stress. The subjects in the study group demonstrated increased levels of salivary alpha-amylase and stress-associated oral diseases.

Keywords: Biomarker; Corporate employees; Psychological stress; Salivary alpha-amylase; Stress-related oral diseases

1. Introduction
Stress has become an imminent part of our busy lives in today’s world. Stress is defined as the psychological and physical state that results when the resources of the individual are not sufficient to cope with the demands and pressures of the situation [1]. Psychological stress has been associated with negative health consequences like Cardiac diseases, Cancers, Gastrointestinal disorders, headaches as well as Oral diseases [2]. People working in the corporate sectors have higher levels of stress and job stress has been a relatively neglected area of research. The demanding framework of the corporate field makes stress at the workplace unavoidable [1]. In India, the software industry has become one of the fastest-expanding industries. The employees of these industries are prone to develop numerous health problems owing to continuous physical and mental stress due to a variety of stressors like extreme competition, unachievable targets, work timings, unfair distribution of work, working relations, etc [3,4]. The assessment of stress can be made qualitatively with the help of questionnaires and quantitative evaluation can be made through various Biomarkers [2]. Several questionnaires like the Perceived stress scale(PSS), Depression Anxiety and Stress Scale(DASS), the State-Trait Anxiety Inventory(STAI), and Hospital Anxiety and Depression Scale(HAD) are used in the medical field. The HAD scale is a routinely used tool to measure anxiety and depression, which is simple and easy to use. It is useful for the initial diagnosis of psychological diseases and has been utilized in many studies [5]. Saliva provides an optimum and non-invasive biological source for quantitative and qualitative assessment of mediators associated with stress. The various salivary biomarkers are Chromogranin A, salivary alpha-amylase, salivary IgA, Cortisol, and Lysozymes [6]. In stress research, Cortisol has become the "gold standard" biomarker to evaluate the dysregulation of the HPA axis and it has widely been studied. Recently, Salivary alpha-amylase has emerged as a valid and reliable marker of Autonomic nervous system (ANS) activity. Together these systems interact with the immune system and are consequently closely involved in the pathogenesis of stress-related diseases [7]. Therefore, Salivary alpha-amylase( sAA) may be utilized as a biomarker for objective evaluation of the psychological conditions of individuals working in stressful surroundings [8]. More studies involving it need to be conducted to authenticate these findings further. The oral mucosa is highly responsive to psychological influences. The oral diseases that are known to occur due to stress are Recurrent aphthous stomatitis(RAS), Oral Lichen Planus(OLP), Xerostomia, Burning Mouth Syndrome, Bruxism, and Myofascial pain dysfunction syndrome(MPDS) [9,10]. Several studies have been conducted which have assessed and associated professional stress with general diseases. But, very few studies have been conducted in the purview of stress-related oral manifestations in the corporate population.
Therefore, the aim of the present study was to first assess the stress levels and salivary alpha-amylase levels of Corporate employees. The study also aimed at observing the prevalence of stress related oral disorders and identifying any correlation between the stress levels, salivary amylase levels and associated oral disorders.

2. Materials and Methods

This study was conducted among 80 patients aged between 25-60 years working in corporate sectors as software engineers or higher cadres, with a minimum of 3 years of experience in the same organization, who visited the outpatient department of Oral Medicine and Radiology, The Oxford Dental College, Bangalore. Subjects were explained in detail about the study procedure and written informed consent was obtained before including them in the study. The ethical clearance was obtained prior to conducting the study from the Institutional Committee and Review Board of The Oxford Dental College, Bangalore. The study was conducted in accordance with the Helsinki Declaration, 1975.

2.1 Inclusion criteria

Men and women who were between 25-60 years of age working in corporate sectors as software engineers or higher cadres and who had a minimum of 3 years of experience in the same organization.

2.2 Exclusion criteria

Participants with conditions that alter salivary amylase levels and those with systemic diseases that cause oral changes as enlisted below were excluded from the study.


A total of 80 subjects were recruited for the study. The sample size was determined based on the power of the study and the confidence interval of 90% (p<0.05). The subjects were included in the study according to subjective evaluation of their stress levels assessed through the Hospital Anxiety and Depression scale (HADs) questionnaire which was used as a screening tool. According to the questionnaire, scores of 0-7 are considered normal, 8-10 are borderline and above 11 indicate "high stress." As per the scores, the subjects were divided into the following groups.

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.

Equal composition of both genders was ensured to facilitate the evaluation of gender-based differences in stress, salivary alpha-amylase levels, and oral lesions. A precise case history was recorded which included the patient's demographic data, medical and dental history. This was followed by a clinical examination of the subjects to detect stress-related oral mucosal changes like Recurrent aphthous ulcers, Oral Lichen planus, Dry mouth, and Burning mouth syndrome. Examination of TMJs, masticatory muscles, and trigger spots was carried out to record Temporomandibular joint disorders(TMDs) which are also related to stress. All the findings were documented in the proforma. Unstimulated salivary samples (1 ml) were collected in sterile saliva collecting containers by the SPIT METHOD. Patients were advised to refrain from intake of any food or beverages at least one hour before the
collection of saliva. All salivary samples were collected from the subjects between 10-11 AM to avoid diurnal changes of Salivary alpha-amylase. The samples were stored at - 20 degrees and were later subjected to Enzyme-Linked Immunosorbent Assay (Human AMYLASE 1 ELISA Kit) for the estimation of Salivary alpha-amylase levels. After estimation of amylase levels, the statistical correlation of salivary alpha-amylase levels with stress levels and prevalence of oral disorders between the groups was analyzed.

2.3 Statistical analysis
Chi-Square Test was used to perform the gender-based comparison of Stress levels and prevalence of associated oral disorders among Study groups and between genders in the study groups. Mann Whitney Test was used to compare the mean Salivary Amylase levels (ng/ml) between Study & Control groups and also between genders in each group. Similar comparisons were performed for mean Salivary Amylase levels (in ng/ml) based on different stress levels between genders and finally between different associated oral disorders in the control group. Kruskal Wallis Test followed by Mann Whitney post hoc test was used to compare the mean Salivary Amylase levels (in ng/ml) based on different stress levels in the Study group and between different associated oral disorders in the study group. The level of significance was set at P<0.05.

3. Results
The study consisted of 80 subjects with an age range of 24-47yrs 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. Among the females in the study group, 5% (N=1) had mild stress levels, 55% (N=11) had moderate stress levels and 40% (N=8) had severe stress levels(p=0.10) (Graph 1). The mean Salivary amylase level in the Study group was 111.943 ng/ml and in the Control group it was 37.597 ng/ml (<0.001). Comparing the salivary amylase levels of males and females in the Study group, it was noted that the mean salivary amylase level in the males was 94.146ng/ml and in the females, it was 129.741 ng/ml (Graph 2). On multiple comparisons of mean Salivary alpha-amylase levels based on stress levels, subjects with moderate stress levels showed higher salivary amylase levels than those with mild stress levels (Graph 3). The subjects with severe stress levels had still higher salivary amylase levels than those with both mild and moderate stress levels(p<0.001). On comparing the prevalence of associated oral disorders between the Study and Control group, it was noted that 17.5% (n=7) presented with RAS, 7.5% (N=3) with Dry mouth, 5% (N=2) with TMDs, and 2.5% (n=1) presented with Dry mouth and TMD and in the Control group, 5% presented with RAS only. These differences were statistically significant (Table 2). The associated disorders were more prevalent in females than males. The mean differences in the salivary amylase levels based on the associated oral disorders both in the Study and Control group were not statistically significant indicating there was no clear association between the amylase levels and oral disorders (Table 3).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Study group</th>
<th>Control group</th>
<th>P-Value</th>
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<tr>
<td></td>
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<td>SD</td>
<td>Mean</td>
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<td>5.88</td>
<td>31.93</td>
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<td></td>
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<tr>
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<td>50%</td>
<td></td>
<td>50%</td>
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</table>

Table 1: Age and gender distribution among two groups

<table>
<thead>
<tr>
<th>Associated disorder</th>
<th>Study group</th>
<th>Control group</th>
<th>$\chi^2$ value</th>
<th>P-Value</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS</td>
<td>7</td>
<td>17.50%</td>
<td>2</td>
<td>5.00%</td>
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<tr>
<td>Dry Mouth</td>
<td>3</td>
<td>7.50%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>TMD</td>
<td>2</td>
<td>5.00%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>Dry Mouth + TMD</td>
<td>1</td>
<td>2.50%</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>None</td>
<td>27</td>
<td>67.50%</td>
<td>38</td>
<td>95.00%</td>
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</table>

Table 2: Comparison of prevalence of associated oral disorder between Study & Control groups

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<thead>
<tr>
<th>Group</th>
<th>Disorders</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>RAS</td>
<td>7</td>
<td>129.153</td>
<td>56.315</td>
<td>82.34</td>
<td>235.67</td>
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<tr>
<td></td>
<td>Dry Mouth</td>
<td>3</td>
<td>130.203</td>
<td>53.195</td>
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<td>191.4</td>
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<tr>
<td></td>
<td>TMD</td>
<td>2</td>
<td>79.365</td>
<td>7.46</td>
<td>74.09</td>
<td>84.64</td>
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<td></td>
<td>Dry Mouth + TMD</td>
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<td>73.86</td>
<td>.</td>
<td>73.86</td>
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<td></td>
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<td>109.276</td>
<td>59.439</td>
<td>53.42</td>
<td>247.76</td>
<td>0.25</td>
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Table 3: Comparison of mean Salivary Amylase levels based on associated oral disorders in Study group using Kruskal Wallis Test
Graph 1: Distribution of stress levels among subjects in Study group

Graph 2: Mean Salivary Amylase levels (ng/ml) between Study & Control group

- **Males**
- **Females**
4. Discussion

Psychological stress is an impending facet of life. In 1996, the World Health Organization had reported that by 2020, Stress-related mental disorders would be highly prevalent [11]. Persistence of stress has been theorized to be a major factor contributing to changes in neuroendocrine, cardiovascular, autonomic, and immunological functioning, leading to various diseases that also include stress-related oral manifestations [1]. Stress has been posing threat to mental and physical health of individuals involving almost all professions. Curiosity in professional stress research is growing essentially because of the increasing incidence of the unfavorable effects of the profession on the psychological as well as the physical health of employees. A study on stress among Indian software professionals revealed that subjects who were professionally stressed had a 10 times higher risk for developing psychiatric disorders and health issues compared to those who were not professionally stressed [1,3,5,12]. Thus, psychological stress which is contemporary and unavoidable in the Corporate sector needs to be addressed and studies conducted at various levels involving this population need to be encouraged. The present study applied the HAD Scale to assess the psychological status of the subjects. The scale was conceived by Zigmond Snaith to measure anxiety and depression in patients which has presently become a favored tool in clinical practice and research. Several studies have proved the reliability of HADS in the assessment of anxiety and depression [5]. A study by Ajmal Muhammed in 2018 found that the HAD Scale worked well in assessing the symptoms and severity of anxiety disorders and depression in patients, and in the general population [13]. The HAD Scale was further used to categorize the subjects into those with mild, moderate, and severe stress levels according to the scores. It was observed that females had higher stress levels than males. This could be possibly ascribed to the fact that most females are primary caregivers, have to multitask, and also play their professional and personal roles laterally. These findings were following a study by Rani and Bhuvaneshwari K, wherein it was found that working women in the age group of 29-32 years had high levels of stress [14]. Since questionnaires are highly subjective in nature, they alone cannot be relied on to assess the stress levels. In our study, the stress levels were also correlated with salivary alpha-amylase which is postulated to be a biomarker of stress. In recent years there has been increasing interest in salivary

Graph 3: Mean Salivary Amylase levels (in ng/ml) based on associated oral disorders in Study group
biomarkers and their role in diagnoses. Compared to cortisol it appears to be uniquely sensitive to interventions that promote better health [7]. Evidence from studies show that changes in the salivary amylase levels are more accurate indicators of an individual's response to stress compared to those of cortisol [8]. Compared to heart rate and systolic blood pressure, salivary amylase is a more sensitive measure of the sympathetic system [15]. Salivary amylase, therefore, appears to be a promising marker for future stress-related studies. For evaluation of salivary alpha-amylase, about one ml of saliva was collected from each subject using the SPITTING METHOD. In our study, the mean salivary alpha-amylase levels (111.943 ng/ml) in the study group were observed to be markedly higher than the control group (37.597 ng/ml) which were statistically significant. Within the study group, the subjects with severe stress levels had correspondingly high levels of amylase which was also statistically significant. This shows a positive correlation between salivary amylase and stress levels suggesting the utility of salivary amylase as a biomarker of stress. A pharmacological study by Stegeren AV provides evidence for the sensitivity of salivary amylase to changes in the adrenergic activation, specifically in reaction to psychological stress [15]. Rashkova Maya et al. also found a significant correlation between salivary amylase and stress levels [8]. In the present study, the amylase levels were higher in females than males in the study group which was statistically significant. This observation is in accordance with a study by Carr AR et al. who found that women are more reactive to stressors than men wherein women displayed a significant increase in salivary amylase from pre- to post-stress [16]. Oral diseases may arise as a direct expression of emotions or indirect result of psychological alterations [17]. The various stress-associated disorders are RAS, OLP, BMS, Dry mouth, Atypical facial pain, TMDs, etc. In this study, it was observed that the prevalence of stress-related oral diseases was higher in the study group in comparison with the control group and the encountered disorders were RAS (17.5%), Dry mouth (7.5%), and TMDs (5%) which were found to be statistically significant (p=0.03). Many studies point out an association between psychological factors and alterations of the oral mucosa [9,13,18-21]. Gender-wise, there was no significant difference in the prevalence of oral diseases. The test results showed that the mean difference in the salivary amylase levels based on the associated oral disorders in the Study group and Control group was not statistically significant which indicated that there was no clear association between the salivary amylase levels and oral disorders. This was in accordance with a study by Vineetha R et al. who found no correlation between amylase and prevalence of oral lesions [2]. Among the prevalent oral diseases, RAS was the most common finding in the study group (17.5%). RAS has multifactorial pathogenesis which includes stress as one of the precipitating factors. Psychological stress induces deregulation in the mechanisms of oral mucosal response against exogenous or endogenous antigens. There is an imbalance of TCD4+ cells with the proliferation of TCD8+ lymphocytes, mediators of cytotoxic reaction which bring about the oro-mucosal alterations [19]. It normally occurs in the younger age group and is more frequently seen in females. In our study, there was no significant difference gender-wise which is in contradiction with several studies which suggest that RAS is predominantly seen in females. There is evidence from many studies that stress is a contributory factor in the pathogenesis of RAS [9,10,13,17]. Gallo CB et al. found that stress may play a role in the manifestation of RAS and may serve as a trigger or modifying factor [19]. Dry mouth was the other disease that was prevalent (7.5%) in the study group which was exclusively seen in females. Several studies have found an association between stress and Dry mouth. The
activity of salivary glands is under the control of the autonomic nervous system. Psychological factors are known to affect saliva composition and salivary protein output. During psychological stress, there is an activation of the sympathetic nervous system by which there is a decrease in the salivary flow leading to xerostomia. The predilection is for females in higher age groups which are similar to the present study. The findings of our study were also in accordance with a study by Gholami Nadi et al. who suggested a correlation between stress, anxiety, depression, and xerostomia [20]. TMDs (5%) were also noted in the study group. Stress has been associated with clenching and grinding of teeth which results in spasm of muscles of mastication which could lead to TMDs [10]. The other reason postulated could be due to ergonomics owing to the wrong posture adopted by the corporate sectors while being stationed in front of the devices. Our observations were in accordance with a study by Al-Khudairy MW et al. who reiterate the role of stress and headaches with TMDs [21]. In our study, other stress-related oral diseases like Burning mouth syndrome and Oral lichen planus were not observed, which could be due to the small size. This study was not without limitations. The sample size was small, so the study results cannot be generalized to the target population.

**Future recommendations**
Since this was an exploratory study it is advocated to conduct, more studies involving larger sample sizes, comparative studies with professionals from various sectors and to explore the prognostic value of Salivary alpha-amylase.

**5. Conclusion**
In conclusion, within the limitations of this study, it can be postulated that increased levels of stress were noted that correspondingly correlated with high levels of Salivary alpha-amylase among the study group participants and also it was identified that there was positive correlation between stress levels and oral changes. However, there was no correlation between the Salivary alpha-amylase and associated oral changes.

**References**
8. Rashkova MR, Ribagin LS, Toneva NG. Correlation between salivary α-amylase and


