ORIGINAL ARTICLE

Aphthous ulcers, salivary peroxidase and stress: Are they related?

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ABSTRACT

Background: In today's high strung lifestyle, stress plays a major role on our health. Studies using ultraweak chemiluminescence have been able to demonstrate this effect, of psychological stress on the immune system, using saliva as a psychological stress marker. The impact of psychosocial factors on the oral mucosal lesions of individuals found that stress can contribute to weakened immunity and increased susceptibility to infection. **Aim:** To study the role of salivary peroxidase (SPOx) in psychologically stressed individuals with and without the presence of aphthous ulcer. **Materials and Methods:** The study involved evaluating subjects for stress, using Perceived Stress Scale. Depending on the stress scores and the presence or absence of oral aphthae, they were assigned into 3 groups of 30 each. After a thorough oral examination, individual samples of saliva was collected and subjected to microprotein estimation using a biochemical analyzer. **Statistical Analysis Used:** Analysis of variance (ANOVA) and Student's *t*-test. **Results:** Decreased levels of peroxidase were found in individuals' with aphthous ulcers, while the same was increased when no lesions were found and also on a lower stress scale. **Conclusions:** Our study analysis does show a variation in enzyme levels between the different groups highlighting the influence of stress on the peroxidase levels, which in turn when imbalanced, results in tissue damage, leading to aphthous formation.

Key words: Aphthous ulcers, perceived stress scale, psychological stress, reactive oxygen species, salivary peroxidase levels

INTRODUCTION

In today's high strung lifestyle, stress plays a major role on our health. The stress system coordinates the adaptive response of the organism to stressors of any kind.[1] The hypothalamus-pituitary-adrenal (HPA) axis becomes active in response to stress. Hence, increased levels of persistent anxiety problems have been associated with changes in HPA axis functioning.[2] Stress may influence immunity either through direct innervations of the central nervous system and immune system (nerves terminating in lymphoid organs) or through neuroendocrine immune pathways (release of hormones). Even behavioral changes occurring as adaptations or coping responses to stress, such as increased smoking, drinking and changes in diet might also influence the immunity and it also depends on exposure to pathogenic agents, as well as period of exposure.[3]

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In a report,[4] regarding the impact of psychosocial factors on the oral mucosal lesions of individuals, it was found that stress can contribute to illness by causing the mind and body to become exhausted, worn down and damaged, thereby weakening the immunity and increasing the susceptibility to infection.

Stress leads to oxidative stress by producing free radicals such as reactive oxygen species and reactive nitrogen species. Reactive oxygen species is a collective term which includes oxygen-derived free radicals such as the superoxide radical (O_2^-), hydroxyl radical (OH) and nitric oxide radical species and nonradical derivatives of oxygen such as hydrogen peroxide and hypochlorous acid.

In the oral cavity, several pathologies have been associated with stress. These include conditions such as periodontal disease, acute necrotizing ulcerative gingivitis, dental caries, recurrent

aphthous ulcerations and upper respiratory infections. These may in turn be due to the aggregation of bacteria in saliva.[5]

The oral cavity contains a plethora of specific and nonspecific defense factors. Nonspecific factors include mucins, proline-rich proteins, salivary glycoproteins, lactoferrins, lysozyme, histatins, cystatins and peroxidases. Amongst them, the innate host defense system consists of peroxidase enzymes and lysozyme.[6] Superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, peroxonase, peroxidase, etc., are the enzymes which are considered as psychological stress markers.

The peroxidase system in saliva comprises of three components:[7]

- The peroxidase enzymes (glycoprotein enzyme), salivary peroxidase (SPOx) from major salivary glands and myeloperoxidase (MPO) from polymorphonuclear leukocytes, filtering into saliva from gingival crevicular fluid
- Hydrogen peroxide, which has three sources; one as an endogenous source of H_2O_2; second from the bacteria during anaerobic glycolysis and finally from activated neutrophils during oxidative burst
- An oxidizable substrate such as the pseudohalidethiocyanate (SCN-) derived from diet and cigarette smoke.

Most of the SPOx activity is associated with the soluble portion of saliva, whereas most of the MPO activity is associated with sediment.[6] Consumption of H_2O_2 is biologically and clinically significant because H_2O_2 is highly toxic to many mammalian cells including fibroblasts and epithelial cells. Thus, the conversion of H_2O_2 and SCN- by salivary peroxidise (SPO) into OSCN-, O_2 and water, abolishes the cytotoxicity, of bacterial H_2O_2 in whole saliva and also SPO prevents the production of H_2O_2 from bacterial cell, thus preventing toxic damage to cells.[8]

Many studies in the past have proved the association of stress to the occurrence of recurrent aphthous stomatitis. It has been suggested that stress along with its presumed effects on the immune system, constitutes one of the major causative agents of RAS.[9]

Literature search showed that the majority of studies have evaluated independently, the association of stress with oral ulcers and the presence of recurrent aphthae to the levels of peroxidase enzymes, while no studies correlated the association of stress, peroxidase enzyme and oral aphthae.

Hence, an attempt was made to compare the levels of SPOx in psychologically stressed individuals with and without the presence of aphthous ulcers, which would throw more light on the role of psychological stress and salivary enzymes, in the etiopathogenesis of oral aphthae.

SUBJECTS AND METHODS

The subjects were selected from the outpatient department of ourinstitution as well as student volunteers. Both the sexes were included in the study. Subjects in the age group 18–25 years and nonsmokers were selected. Subjects with history of tobacco usage, pregnant and lactating women and with any history of systemic diseases and current medication use, were excluded from the study.

Using the Perceived Stress Scale, each individual was scored on a predetermined scale and the results obtained were statistically analyzed using analysis of variance (ANOVA) and *t*-test.

Individuals were screened and evaluated and those who satisfied the criteria were grouped as shown in Table 1.

The procedure as stated by Kanehira *et al*.,[9] was followed, where the participants refrained from consuming any food or beverages 2 h prior to collection of saliva in the morning. Following a thorough mouth rinse using distilled water, approximately 2–3 ml of nonstimulated whole saliva was collected for 20 min (splitting method).

The collected saliva sample was centrifuged at 6,500 rpm for 10 min at 4°C to remove microorganisms, saburra and desquamated epithelial cells. The resulting supernatants were analyzed for SPOx activity following the method of Pruitt *et al*.,[10] using an automated biochemistry analyzer for microprotein value estimation. Finally, the specific activity of the enzyme was expressed as units per milligram of protein in saliva. The mean values of the data were compared between the groups using the Student's *t*-test and ANOVA.

RESULTS

It was found that the salivary peroxide levels were highest in the group that had no oral aphthae, while the peroxide levels dropped once the lesion were established. Also it was found that the SPOx levels were higher in males between the groups. The results of which are summarized in Tables 2 and 3 and Figure 1.

DISCUSSION

Stress is considered as a most important etiological and risk factor for many diseases in the recent times. Stress has been

Table 1: Categorization of participants

	Group I	Group II	Group III
Groups	Stress with oral aphthae	Stress without oral aphthae	Controls
No. of individuals	30 (M-7; F-23)	30 (M- 09; F-21)	30 (M-11; F-19)

M: Male, F: Female

Table 2: The mean of individual groups of stress was calculated and further comparison of the total number of individuals resulted in the respective F-values, which were significant

	Group I		Group II		Group III		F-value	*P* value
	Mean	SD	Mean	SD	Mean	SD		
Total group	22.0	3.92	20.1	2.928	13.06	2.612	66.08	0.0001 (significant)
Females	22.428	4.1901	20.157	3.4843	14.29	1.6494	28.41	0.0001 (significant)
Males	21.222	3.3829	20	1.7320	11.46	2.8170	45.54	0.0001 (significant)

SD: Standard deviation

Table 3: Comparison of SPOx enzyme levels among three groups showing the f-values, with a significant difference among the males of the three groups

	Group I		Group II		Group III		F-value	*P* value
	Mean	SD	Mean	SD	Mean	SD		
Total group	0.687	0.767	1.1936	0.933	1.079	0.999	2.546	0.084 (not significant)
Females	0.8111	0.8903	1.3252	1.1116	0.8957	0.8299	1.616	0.208 (not significant)
Males	0.4002	0.1433	0.9661	0.46	1.3178	1.1783	3.549	0.041 (significant)

SD: Standard deviation, SPOx: Salivary peroxidase

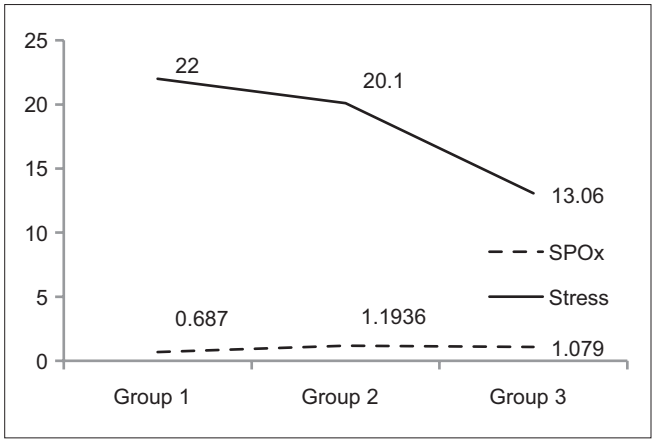

Figure 1: Correlation between mean stress and SPOx levels among three groups. SPOx = Salivary peroxidase

associated with many lesions/conditions affecting the general health of an individual and has time and again been associated with oral lesions.The effect of psychological stress on the oxidative stress hasbeen reflected by the antioxidant and antimicrobial activity of saliva, where many of the salivary enzymes are considered as stress markers.

Psychological stress induces immunoregulatory activity by increasing the number of leukocytes at sites of inflammation, which is often observed in RAS. Some investigators also speculated that anxiety could lead to parafunctional oral habits, including lip and cheek biting and that physical trauma may initiate the ulcerative process in susceptible individuals.[11]

The SPOx system constitutesSPOx (secreted by salivary glands), hydrogen peroxide (derived from bacteria, leukocytes and host cells) andthiocyanate (derived from diet and saliva) whichact in preventing a toxic accumulation of 'hydrogen peroxide', thereby regulating the growth and metabolism of oral bacteria and also inactivating many carcinogenic and mutagenic compounds.[12] The accumulation of hydrogen peroxide above the physiological values results in an oxidative stress that causes the cytotoxic effects on mammalian cells and leads to cell damage through its damaging effects on peroxidation of double chain fatty acids, proteins and DNA. This leads to the occurrence of oral lesions.[13] Oxidative stress is defined as "the imbalance between the production of reactive oxygen and nitrogen species and their insufficient decomposition by the antioxidative system". It appears that imbalance between free radicals and antioxidants causes many inflammatory oral soft tissue diseases varying from infections and immunologic diseases to lethal cancers. Under physiologic conditions, maintenance of a stable state by the enzyme system is referred to as the "redox homeostasis".

Human whole saliva is a mixed fluid comprising secretions from major and minor salivary glands, a serum derived transudation from gingival crevices as well as components from oral microorganisms, leukocytes and epithelial cells. Measurement of SPOx levels in whole saliva gives the normal values; whereas the quantities of SPOx enzyme levels in 'stimulated saliva' are increased. In stimulated saliva parotid secretions, O_2 concentrations sharply decreases, which results in decreased production of H_2O_2 in saliva and this in turn decreases the OSCN-levels.[14] Hence in the present study, whole saliva was collected for the measurement of peroxidase levels from the different study groups.

In this study, saliva was collected 2 h after breakfast, that is, around 10 AM to minimize the circadian rhythm effects. Also oral microorganisms are relatively inactive between the periods of food ingestion. The bacteria become metabolically active, when food is ingested and H_2O_2 is produced by many species, which may have an impact on the peroxidase enzyme levels. The salivary glands gets stimulated during and immediately after the intake of food and salivary secretions are more during this period and the enzyme activity is low in

the stimulated saliva.[12] Therefore a gap of 2 h was given after breakfast, for the collection of saliva in this study.

The stress evaluation in the selected individuals was done using the Perceived Stress Scale. This test was preferred over other methods of assessing stress as it included a number of direct queries about current levels of experienced stress. These questions were of a general nature and relatively free of content specific to any subpopulation group. The scoring was done on a preset level. The perceived stress during the last month reflects any objective events that are still affecting respondents' stress levels. This scale was found to be reliable and valid in many studies.[15-17]

Comparison of stress values between the three groups showed significant difference, the highest mean value of stress in Group I and the lowest in Group III, where Group I had the oral aphthae lesions, thus probably indicating a cause and effect [Table 2].

Comparing the stress values between males and females within the groups, significant difference within Group III was noted, while slightly higher mean values in females were found, which may be attributed to the periodic hormonal changes in females [Table 2].

A variation in the levels of the SPOx was noticed between the three groups [Table 3], the highest in Group II, followed by Group III and Group I, though not statistically significant.

Also, no significant difference in the levels of enzyme was seen between the genders, within the groups, similar to a study by Kraus *et al*.,[18] who stated that an equal activity of peroxidase enzyme in both sexes could also be found.

But comparing the level of SPOx levels between individuals of Groups I and II, a statistical significance was observed [Figure 1]. Though both the groups contained stressed individuals, the group without the oral lesions (Group II) showed an increased enzyme levels which possibly reflects a compensatory mechanism of the body to prevent oxidative damage.[19] But when the stress levels continue to increase, the body's regulatory mechanism fails, leading to an imbalance in the oxidant/antioxidant activity, resulting in decreased enzyme levels and in turn tissue damage,[13] as seen in Group I individuals, resulting in oral aphthae. This correlation of increased peroxidase enzyme levels and tissue changes in early lesions and its decreases once the lesions are established has been documented by Smith *et al*.,[20] and Guven *et al*.,[21] similar to our finding.

Though variation of enzyme levels existed in the both the genders, a significant level of difference was noted only inbetween the groups of male population which probably might be due to the higher stability of oxidant/antioxidant status in females owing to the hormone regulatory mechanisms.

Based on our findings, we would like to point to the fact that, the mean levels of peroxidase enzyme showed a definite variation, though not statistically significant, indicating the influence of peroxidase enzyme to that of occurrence of aphthous ulcers. The association of stress was found to be higher in individuals with aphthous ulcers, probably pointing to a cause and effect relationship, suggesting a positive correlation between increased stress, decreased peroxidase levels and occurrence of aphthous ulcers. The results therefore suggesting, the need to look at oral lesions with a broader prospective, beyond local factors, which would help understand such lesions better and bring about treatment alternatives.

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