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SALIVARY NITRIC OXIDE AND SALIVARY ALPHA AMYLASE AS BIOMARKERS IN PATIENTS WITH AND WITHOUT OLP –A BIOCHEMICAL STUDY.

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ABSTRACT In the scope of the study with respect to the salivary biomarkers, Salivary alpha amylase and Nitric oxide, significant differences were found between patients with and without Oral Lichen Planus. Overall results also suggest that Salivary amylase and Nitric oxide levels may be correlated to the stress in the subjects with Oral lichen Planus. Salivary alpha amylase and salivary nitric oxide levels increase in patients of Oral Lichen Planus and stress and can be used as a biomarker in stress, however, it may not be an indicator to suggest the development of stress related oral mucosal changes. The severity of stress suffered by each patient in study group was not considered in the study. Whether higher levels of these markers in the patients was a reflection of their chronic stress status or was due to the acute exacerbation could not be differentiated. Even though the sample size in our study was statistically sufficient, study using a larger sample size is recommended to confirm our findings and substantiate the results. 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 and salivary nitric oxide among the study group. Lastly, not many studies have been reported in the literature, which provide information about the usefulness of salivary alpha amylase and salivary nitric oxide in chronic psychological stress, further research in this aspect in comparison with various biomarkers is warranted to establish our findings.

KEYWORDS :

INTRODUCTION

OLP is an immunologically mediated inflammatory response. The basal epithelial cells are the target for immune destruction by cytotoxic T lymphocytes. Excess nitric oxide (NO) is produced via expression of inducible nitric oxide synthase (iNOS) one of the type of nitric oxide synthase. Overproduction of NO leads to generation of various RNS. RNS can mediate the formation of 8-nitroguanine, a marker of nitrative DNA damage. This DNA damage could possibly imply an association between the existence of OLP and the development in to oral cancer.[1]

The salivary alpha-amylase enzyme (SAA) is produced by the salivary glands and its main function is to initiate the digestion of macromolecules such as carbohydrates. Its release is regulated by the sympathetic autonomic nervous system (SANS), whose action is of paramount importance in the psychobiology of stress. Studies show that the SAA levels in humans increase under physical and psychological stress. For this reason, the SAA has become a marker of stress and anxiety, being a fast, painless, and non-invasive tool for assessment of the ANS pathological dysregulation in specific clinical and subclinical conditions.[2]

The literature reports increased levels of SAA under various conditions of physical and psychological stress. Overall, the studies evaluate the activity of this enzyme in response to acute stress, that is, immediately after stressful situations or tasks. In these cases, there is a significant increase in the SAA activity as an immediate response to the stressor agent.[2]

Although there has been considerable research in identifying Nitric oxide and Salivary alpha amylase as biomarkers for both stress and lichen planus, studies about their correlation and comparision is scarce, in view of this, our study with the title " Salivary Nitric Oxide and Salivary Alpha Amylase as biomarkers in patients with and without Oral Lichen Planus: A Biochemical Study " was designed to investigate whether or not a significant correlation exists between the two.

The purpose of the present study were to evaluate the salivary NO & SAA levels and psychosocial stressors and to correlate them in pathogenesis of OLP.

MATERIALS AND METHODS

The present study was carried out in the Department of Oral and Maxillofacial Pathology, Divya Jyoti College of Dental Sciences and Research. The study group comprised of total 100 patients. Subjects were divided into 2 groups,

Group A (Control Group)- 50 normal healthy subjects having no orodental complaints.

Group B (Study Group)- 50 confirmed cases of Oral Lichen Planus.

The study was approved by the Institutions Ethical Committee.

The psychometric evaluation was done using DASS-21.This scale was used to check the levels of stress in individuals with OLP.[3] Saliva samples were stored in ice to prevent loss of nitric oxide. In the Eppendorf tube, 0.75 ml cold absolute ethanol was added to 0.75 ml saliva then was left for 48 h in the refrigerator to attain complete protein precipitation.The mix was then centrifugated at 4000 rpm at 12º C for 30 min using cooling centrifuge (Remi, India). Only 250 ul of the obtained supernatant was used to which 250 ul vanadium chloride (Qualigens) was added followed by rapid addition of 125 ul sulfanilamide (1%) in 5% HCl and 125 ul of N-(1-Naphthyl) ethylenediamine dihydrochloride (0.1%) in distilled water.The mixture was left at room temperature for 30 minutes then the absorbance of the chromophore was measured in a spectrophotometer which was connected to a computer to record the digital values.In a similar manner ,samples of 50 patients for salivary alpha amylase were collected and 1 ml from each sample was transferred to into a test tube, 1 mL of 1% soluble starch in citrate-phosphate buffer (pH 6.5) was added. The mixture was Incubated in a water bath at 400C for 30 minute and treated with the same reagent as the experimental tubes. 2 mL of DNS reagent is added and boiled for 5 minutes. Colour intensity is determined at 540 nm by Spectrophotometer. Salivary amylase levels were estimated with a biochemical analyser, recorded and statistically analysed for results.

RESULTS

Fifty OLP patients were subjected to measure NO and SAA levels. The

mean Salivary nitric oxide level based on Griess method was 62.30 µM/ml in subjects with Oral lichen Planus with a standard deviation of 1.06 µM/ml , in contrast to subjects without Oral lichen Planus where mean value was 8.35 µM/ml. The mean Salivary amylase level based on DNS method was 182.54 U/ml with standard deviation of 0.93 U/ml in subjects with Oral lichen Planus and a mean of 51.38 U/ml in subjects without Oral Lichen Planus,however, the results were statistically insignificant.

Table 1. shows comparision Of Nitric Oxide levels between subjects without Oral lichen Planus, control group (Group A) and subjects with lichen planus (Group B)

Table 1

	Minimum	Maximum	Mean	Std. Deviation	P value	Signi-ficance
Control (Group A)	20.70 U/ml	80.20 U/ml	51.38 U/ml	16.03 U/ml	0.001	Signi-ficant
Lichen Planus (Group B)	180.60 U/ml	184.10 U/ml	182.54 U/ml	0.93 U/ml		

Table 2. showing Intergroup Comparison Of Salivary Amylase Levels Between subjects with Oral Lichen Planus (Group B) and subjects without Oral Lichen Planus, control group (Group A).

Table 2.

	Minimum	Maximum	Mean	Std. Deviation	P value	Significance
Control (Group A)	4.30 µM/ml	13.00 µM/ml	8.35 µM/ml	2.30 µM/ml	0.001	Significant
Lichen Planus (Group B)	60.10 µM/ml	64.40 µM/ml	62.30 µM/ml	1.06 µM/ml		

DISCUSSION

Lichen planus (LP) is a chronic inflammatory mucocutaneous disorder with varied clinical presentation, most frequently as reticular white patches or erosive areas on the oral mucosa which may persist for many years. Histological criteria for Oral Lichen Planus are epithelial hyperkeratosis, acanthosis or atrophy, degeneration and destruction of the basal epithelial cells, and thickening or destruction of the basement membrane. A band-like infiltrate of mononuclear cells in the upper corium, and variable numbers of intraepithelial mononuclear cells closely associated with the basal keratinocytes, are also seen.[4]

Nitric oxide (NO) is a highly reactive free radical composed of one atom of nitrogen and one atom of oxygen. It is synthesized from substrate amino acid, l-arginine, by the enzyme nitric oxide synthase (NOS). It reacts with super oxides to form oxidant peroxynitrate which is responsible for cell injury. The enzyme NOS is capable of producing Nitric Oxide over a longer period of time by immunological mediators and cells including macrophages, T-cells, and natural killer cells. This study was aimed to estimate the level of salivary Nitric Oxide in patients with Oral lichen planus.[5] In the present study, the salivary Nitric Oxide levels in patients with Lichen planus ranged from 60 to 65 µM/ml. [Table 1.] In normal group, salivary Nitric Oxide levels ranged between 5 to 13 µM/ml. The values of Nitric Oxide in the study were significantly raised when compared with that of normal population. This was similar to the findings reported by Sunitha et al., (2006) who concluded that the pathophysiology of Oral lichen planus was associated with an increase in Nitric Oxide levels in saliva. [6]

The salivary nitric oxide levels was significantly higher in subjects with Oral Lichen Planus (p=0.001). It may be likely that Nitric oxide over expression may be correlated to increased prevalence of Oral lichen planus. Increase in salivary alpha amylase during psychosocial stress may be explained on the basis of physiological response to stress. Shyuichi et al., (2007) have tried to correlate salivary alpha amylase levels with pain scale of patients with chronic pain. They found a significant correlation with pain intensity and salivary alpha amylase (p<.01) and suggested it as a good index for measuring pain intensity. There is a close association between salivary alpha-amylase and plasma norepinephrine under stressful conditions. In this study, we tried to determine the usefulness of salivary alpha-amylase as an objective biomarker of stress. The mean salivary amylase levels based on DNS method was 182.54 U/ml with standard deviation of 0.93 U/ml in subjects with lichen Planus and 51.38 U/ml in subjects without Lichen Planus, control group [Table 2].

Psychosocial stress is widely known to induce various adaptational responses of physiologic systems with particular increasing activities in the hypothalamus-pituitary-adrenal axis (HPA) as well as in the sympathetic-adrenal-medullary (SAM) system. Cortisol levels reflect the HPA activity whereas salivary alpha amylase is said to reflect the SAM activity.[7]

Moncad et al [8]opinioned that increased chronicity (inflammation potential), as well as the release of proinflammatory cytokines (IL-1, TNF. etc.) are key activators of NO (iNOS) leading to the production of NO; which in turn mediate DNA damage directly or indirectly through the generation of more persistent RNS. Ohashi et al [9] also reported that NO increase caused severe damage to fibroblasts, keratinocytes, and oral epithelial cells in vitro. Hence, the increased salivary NO levels can be attributed in infectious or inflammatory conditions, which leads to the overexpression of iNOS; thus an increased NO could lead to an increased cellular infiltrate seen in different forms of OLP.Stress as a concept describes the effects of psychological and environmental factors on physical and mental well being.[10]

CONCLUSION

In present study, we concluded, role of Stress in Oral lichen planus could be established by both Salivary Nitric Oxide and Salivary alpha amylase levels. It may further be hypothesized that the stressors may form a starting point for the initiation of various autoimmune reactions, which have been shown to be contributory to the pathogenesis of Oral lichen planus.

The pathways leading to NO load during chronic stress as well as the pathophysiological role played by NO in accounting neuronal damage has been clearly depicted. Thus it can be concluded that a complex cascade of events involving the above mechanisms may be responsible for the etiological interplay between stress and NO load in specific encephalic regions and precipitation of secondary neurological disorders like stress.

Salivary alpha amylase increases in patients of Oral Lichen Planus and stress and can be used as a biomarker in stress, however, it may not be an indicator to suggest the development of stress related oral mucosal changes. The severity of stress suffered by each patient in study group was not considered in the study. Whether higher levels of these markers in the patients was a reflection of their chronic stress status or was due to the acute exacerbation could not be differentiated.

Even though the sample size in our study was statistically sufficient, study using a larger sample size is recommended to confirm our findings and substantiate the results.

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