Original Article

Correlation between nitric oxide level and stress in Lichen Planus patients

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Abstract

Background: Psychosomatic factors and their association with dermatological disorders are well recognized, yet their importance in oral lichen planus (OLP) is still debated. Aim: To evaluate the salivary NO levels and psychosocial stressors and to correlate them in pathogenesis of OLP. Materials and Methods: The study consisted of two groups: Group-I constituted the subjects with OLP group (n=25) and group II comprised the control group (n=25). The saliva of the patients was evaluated using Griess Reagent and Spectrophotometer, and stress levels measured using DASS Scale. Results: The difference between the means of NO levels was found to be highly significant ($P < 0.05$). The intergroup comparison of optical density (OD) values, and stress level was found to be highly significant. Conclusion: Salivary NO and stress has a definitive role in OLP pathogenesis.

Key words: Depression, anxiety, stress lichen planus.

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Introduction

Oral lichen planus (OLP) is a common, chronic inflammatory mucosal disease affecting the skin, genital mucosa, scalp, nails, and areas in the oral cavity.[1,2] The association of chronic inflammation with cancer has been addressed.[3] Reactive nitrogen species (RNS) are considered to play a key role in inflammation mediated carcinogenesis generating nitric oxide (NO). Nitric oxide, a free radical gas, is a noxious chemical in the atmosphere but in small controlled concentrations in the body, acts as a physiological and pathophysiological mediator. The known biological functions of nitric oxide can acts as an endothelial-derived relaxer of vascular smooth muscle, and secondly, acts as cytotoxic molecule influencing the ability of cells to kill bacteria, viruses, protozoa as well as tumour cells. In addition, it is well established that nitric oxide has damaging effects against cellular proteins, DNA and lipids eventually leading to cell death, tissue injury and organ failure.[4] The purpose of the present study were to evaluate the salivary NO levels and psychosocial stressors and to correlate them in pathogenesis of OLP.

Materials and Methods

The study was conducted in the Department of Biochemistry, using saliva samples of patients who visited the college outpatient department (OPD). The study consisted of two groups: Group I constituted the subjects with OLP group (n = 25) and group II

comprised the control group (n = 25). The consent was obtained from all patients and volunteers for examination. The study was approved by the Institutions Ethical Committee. Patients with age group of 20-45 years confirmed Lichen Planus by clinically and histopathological criteria were selected.
Criteria for selecting the control group (II) Age group same as group I subjects (20-45 years), patients undergoing prophylactic removal of tooth: for orthodontic treatment, impacted third molars, free of any inflammation, and free of any systemic disorders.

Stress was evaluated by using Depression Anxiety Stress Scale (DASS). Analysis of stress by DASS is a promising measure of depression, anxiety, and stress. This scale was used to check the levels of stress in individuals with OLP. These solutions were reacted with Griess reagent which was prepared using 1% sulfanilamide, 1% naphthylethylene diamine dihydrochloride and 2.5% phosphoric acid. Griess reagent is very unstable as it reacts with surface atmospheric nitrogen. Hence it was freshly prepared before use. 0.5 ml of the prepared standard solutions of sodium nitrite were reacted with equal volume of Griess reagent in Eppendorf tubes and incubated at room temperature for 10 minutes to ensure that complete reaction takes place. The reacted mixture was then transferred onto plastic cuvettes for measurement in the spectrophotometer which is connected to a computer so that digital readings can be taken. Using these readings taken for the standard solutions, a graph of absorbance versus concentration was plotted, which constituted the standard curve. In a similar manner, samples of the 50 subjects were reacted with Griess reagent and transferred to spectro-photometer and their optical densities (OD) were recorded. The optical densities were then correlated in the standard curve and corresponding concentrations of nitrite were observed. NO was expressed as µmol/L. Biopsies were taken to diagnose lichen planus. Results obtained were subjected to statistical analysis by using the Statistical Package for Social Sciences (SPSS).

Results

Twenty five OLP patients were subjected to measure NO levels. The mean NO levels in saliva of OLP was observed to be 80.88±16.05 mM/L as compared to mean of 8.82±2.40 mM/L NO levels in healthy control. The intergroup comparison of OD values of the two groups was found to be highly significant. Keeping the salivary NO as constant factor, NO was correlated to the increase in age of the patient using Pearson's correlation; however, the results were statistically insignificant.

Table 1: Assessment of difference in the mean salivary nitric oxide levels between the two groups

Groups	Mean±S.D NO (µM/L)	p value
I	10.22±2.05	0.01
II	6.82±1.40	(p<0.0.5)

The comparison of depression, anxiety, and stress scales between the two groups; group I (OLP) and group II (healthy controls) showed a definitive increase in depression levels between the two groups. Group I with

Table 2(a): Assessment of mean of depression levels between the two group

Groups	Mean±S.D NO (µM/L)	P value
I	80.88±16.05	0.012
II	8.82±2.40	p<0.0.5)

Table 2(b): Assessment of means of anxiety levels between the two groups

Groups	Mean±S.D NO (µM/L)	p value
I	9.80±1.05	0.065
II	7.82±0.40	p>0.0.5)

Table 2(c): Assessment of means of stress levels between the two groups

Groups	Mean±S.D NO (µM/L)	p value
I	10.36±3.05	0.075
II	9.82±2.40	p>0.0.5)

a mean of 9.80±1.05 as compared to mean of 7.82±0.40 in group II, and was statistically significant (P < 0.05); whereas, the anxiety and stress scales of group I and group II were insignificant as well (P > 0.05).

Discussion

Lichen planus has remained matter of debate since its first description by Wilson.[5] On comparison of the salivary NO levels in OLP patients with the control group, in our study we found a significant increase in the levels of salivary NO in OLP patients as compared to the control group (P < 0.05)[6] We hypothesized that T lymphocytes from OLP tissues produced increased levels of interleukin (IL)-6 and granulocyte macrophage colony stimulating factors (GM-CSF), which further produces more TNF-α, IL-1b, IL-6, and GM-CSF which then leads to increased production of NOS responsible for releasing NO. Increased NOS activity interacts with caspase family of enzymes, thereby promoting apoptosis, which was seen as basal cell degeneration in OLP histopathologically. Moncad et al[7] 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[8] 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.[9] Stress as a concept describes the effects of psychological and environmental factors on physical and mental well being.[9] It plays a major role in immunological diseases and immune-related disease processes. Inflammation, infection, autoimmune processes, and perhaps even the onset and development of malignant tumors may occasionally be associated with stress phenomenon. Hence, the role of stress in OLP patients was analyzed using DASS. The results showed a significant increase in depression scale (P < 0.05) [Table 2a] in case of OLP patients as compared to the control group. Though the anxiety and stress scales increased in OLP patients as compared to the control group, statistically the values for anxiety and stress levels in OLP patients was not significant (P > 0.05) McCartan[10] studied the psychological factors associated with OLP. Some patients with OLP suffer from depression while some patients have anxiety. Other authors have also pointed out that more than half of his patients with OLP related high levels of stress in relation to work, relationship, and losses; before or during the appearance of the condition. Stress as a pathological factor in OLP can be explained on the hypothesis that glucocorticoids can affect the lymphocyte subsets and induce a shift between Th1/Th2 cytokines; while preferentially inhibiting nonactivated lymphocytes, thus favoring IL-2 expression

during clonal expansion.[10] Moreover, major depression has been associated with activation of the inflammatory response as proinflammatory cytokines are potent stimulators of neuroendocrinal response.[11] Hence, following conclusions were proposed: Free radical including NO represents one route of pathogenesis and that excess of salivary NO has a pathophysiological implication in OLP. The NO (iNOS) can activate the inflammatory cytokine which has damaging effects against cellular proteins, DNA and lipids eventually leading to cell death, tissue injury and organ failure. Thus, biochemical analysis of patients with OLP can aid to a more improved therapeutic plans, as well as guide in checking the malignant potential of the lesion.[11]

Conclusion: Salivary NO and stress has a definitive role in OLP pathogenesis. It may be further hypothesized that these stressors form a starting point for the initiation of various autoimmune reactions, which have been shown to be contributory to the pathogenesis of OLP. Further longitudinal studies need to be done globally before definitive conclusions can be drawn.

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