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Evaluation of Oxidative stress using Superoxide Dismutase and Lipid Peroxidation in Lichen Planus: A Tissue Level Enzymatic Analysis Study

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Abstract

Introduction: Oral Lichen Planus (OLP) is a chronic inflammatory disease of oral mucosa with unknown etiology. Many studies have suggested that increased oxidative stress, an imbalance in the antioxidant defense system may be involved in pathogenesis of Lichen planus.

Aim: The aim of this study was to compare the tissue levels of antioxidant Super oxide dismutase (SOD) and lipid peroxidation marker Malondialdehyde (MDA) in established cases of OLP and healthy individuals.

Methodology: Ten patients with OLP and 10 control subjects matched for age and sex were enrolled in this case control study. The tissue levels of SOD and MDA were measured both in case and control groups.

Results: The tissue levels of SOD were increased that was statistically significant in OLP patients compared to healthy controls ($p<0.01$). The lipid peroxidation product MDA were relatively higher in OLP patients compared to healthy controls. Conclusion: The current study suggests that oxidative damage is one of the major causes for the pathogenesis of OLP.

Key words: Oxidative stress, ROS, immunological disease, apoptosis

Introduction

Oral Lichen planus is a common disorder of the squamous epithelia. OLP is a T-cell-mediated mucocutaneous chronic inflammatory disease. The exact pathogenesis of the OLP remains unclear, many factors are proposed for the pathogenesis of OLP including Genetic; Bacterial and viral infections; Autoimmune diseases; Immunodeficiency; Dental materials; Diabetes; Hypertension; Drugs; food allergies; Stress and trauma[1,2]. It is hypothesized that both antigen-specific and non-specific mechanisms are involved in the pathogenesis of OLP[3,4].

In immune specific mechanisms there is activation of intra-epithelial CD4+ T helper cells and CD8+ cytotoxic T-cells by lichen planus antigen by MHC class I and II molecules or encountering the antigens in the basal keratinocytes. The lesional keratinocytes secrete cytokines and direct the T cells to migrate into the epithelium and trigger keratinocyte apoptosis[3,5,6].

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The non-specific mechanisms are associated with pre-existing inflammation causing the infiltration of lymphocytes. The in filtered T-cells release activators of zinc containing matrix metalloproteinase (MMPs) and causes the degranulation of mast cells. The principal function of MMPs is proteolytic degradation of connective tissue matrix proteins. This causes the destruction of keratinocytes of the epithelial basement membrane[1,4,7,8].

Both keratinocyte apoptosis and basement membrane disruption may be involved in the pathogenesis of OLP e.g., basement membrane disruption may trigger keratinocyte apoptosis, and apoptotic keratinocytes may be unable to repair the disrupted basement membrane. Such a cyclical mechanism may underlie disease chronicity[2,4].

Both the mechanism produce antibodies and T-cell mediation have been implicated. Activated T-cells release cytokines leading to the attraction of inflammatory cells and the destruction of keratinocyte by cell-mediated cytotoxicity. There are different factors that could play as triggers to induce OLP[9].

Reactive oxygen species (ROS) are involved in the etiology and the pathogenesis of a variety of diseases in which ROS were involved in the initiation stage or are produced during its course[2,6-9]. ROS are continuously produced in aerobic life; they are generated in vivo by multiple mechanisms, including the respiratory redox chain in mitochondria, the respiratory burst of phagocytes, and the activity of various oxidase[10]. The excess ROS are toxic to the cells it can damage cellular lipids, proteins, nucleic acids inhibiting their normal function[11]. To defend such damage the body possesses several enzymatic and non-enzymatic antioxidant system that are important in the prevention of oxidative stress[12]. Imbalance of the oxidant-antioxidant system resulting in excessive accumulation of ROS[10]. Several studies have evaluated salivary and blood level of oxidative stress markers in OLP patients and suggest that oxidative stress is implicated in the pathogenesis of OLP. The purpose of the present study was to evaluate the lipid peroxidation marker malondialdehyde (MDA) and anti oxidant enzyme superoxide dismutase (SOD) in the tissues of OLP to broaden the horizon related to its pathogenesis.

Materials and Method

The study was carried out in the Department of Oral & Maxillofacial Pathology, Faculty of Dental Sciences and Department of Biomedical Research, Sri Ramachandra University (SRU). The study group comprised of 10 subjects with OLP and 10 healthy subjects as the control group. The following inclusion and exclusion criteria were used to select the patients and controls for the study. The diagnosis of lichen planus was done after all patients were subjected to clinical and histopathological examination. Patients were selected based on histopathological evidence of lichen planus in biopsy specimens from the lesions. Subjects using tobacco, alcohol and any other medically compromised illness which may influence the antioxidant status were excluded from the study. The study was approved by the ethical committee of Sri Ramachandra University and informed consent was obtained from all of the participants prior to investigations.

Microscopic examination

Oral biopsies specimens were sectioned using a Semi-automatic Leica microtome and stained using the standard Hematoxylin–Eosin method (HE). Microscopic examination of tissue analysis was performed using Olympus Chi20 microscope equipped with image analyzer software.

Oxidant and Antioxidant Assays

The marker of antioxidant defense, antioxidant enzyme super oxide dismutase (SOD), was estimated based on the photometric determination of Kakkar P et al[13] from the preserved oral tissue. The results were expressed in U/min/mg protein. The oxidative stress marker for lipid peroxidatiion, malondialdehyde (MDA) was assessed based on the photometric method of Ohkawa H et al[14]. The results were expressed in mg/gm of tissue.

Statistical analysis

The data were analyzed statistically using unpaired t-test. The correlations between the qualitative variables were analyzed by chi-square test. SPSS 15.0 statistical software was used to analyze all data.

Results

The average age group of the OLP patients was 45.6 ± 13.01

Histopathological sections showed parakeratotic stratified squamous epithelium with basal cell degeneration. The underlying connective tissue stroma showed juxta epithelial intense chronic inflammatory cell infiltration chiefly lymphocytes, plasma cells & dilated young blood capillaries suggestive of Lichen Planus.

The level of the antioxidant enzyme, SOD among the patients was found to be 5.35 U/mt/mg ptn while that of the controls was found to be 1.004 U/mt/mg ptn (Table 1). The mean expression of MDA among the cases was found to be 44.657 µg/g tissue while that of the controls was found to be 39.33 µg/g tissue.

Median MDA levels were significantly increased in the tested group: 2.67 (0.26–3.40) vs. control group 0.44 (0.19–0.70). The simple comparison between groups has returned a value of 0.021. After age adjustment the difference was highly significant ($p<0.0001$)). GSH medium level was significantly decreased in patients tissue compared to controls: 2.3 (1.25–5.70) vs.9.56 (6.5–12.5). Non-adjusted significance was p=0.005. After the age adjustment, $p<0.0001$ (highly significant).

Table 1: Comparison of mean expression value of SOD among the cases and controls

Parameter	Group	N	Mean	SD	p-value
SOD	Case	10	5.3500	6.97	0.000 (Significant)
	control	10	1.0040	0.399	

p-value is significant below the value of 0.05

Table 2: Comparison of mean expression value of MDA among the cases and controls

Parameter	Group	N	Mean	SD	p-value
MDA	Case	10	44.657	16.214	0.249 (Not significant)
	control	10	39.33	13.369	

p-value is significant below the value of 0.05

Discussion

Oxidative stress may damage cell membranes through production of lipid peroxides, as well as molecules such as nucleic acids, proteins and carbohydrate. To counter the deleterious actions of ROS, antioxidant enzymes are synthesized in response to higher production of ROS. Therefore, a study was undertaken to evaluate the levels of SOD and MDA in the tissue samples of oral lichen planus.

In this study a significant increase in SOD activity was observed in the tissue of Oral lichen planus patients with the mean value of 5.35U/mt/mg of protein as compared to the controls with the mean value of 1.004 U/mt/mg of protein (as shown in Table 1).

Similar studies were conducted by Sezer et al[6], who concluded increase in serum SOD levels in patients with lichen planus as compared to the healthy controls.

SOD constitutes the first line of defense against oxygen derived free radicals by converting $O2^-$ to H2O2. Due to accumulation of H2O2, there is vacuolization of the basal layer in lichen planus.CAT is the main enzyme involved in the removal of H2O2, which is generated by superoxide anion radicals by SOD.

There is an imbalance in the antioxidant status which may lead to accumulation of H2O2, thus leading to basal cell degeneration seen in lichen planus. This is the reason for increased tissue SOD levels in lichen planus as compared to the healthy controls in our present study.

The protein value of the cases and controls were determined to assess the value of Malondialdehyde (MDA), which is a by-product of lipid peroxidation.

Malondialdehyde (MDA), a product of lipid peroxidation induced by reactive oxygen species (ROS) is well correlated with the degree of lipid peroxidation. MDA is an indicator of oxidative stress.

Lipoperoxidation, the primary reaction sites

of which involve membrane associated PUFAs of phospholipids can be a major manifestation of oxidative stress. In this study, although higher levels of MDA in the tissues of patients with Lichen planus (44.65µg/g tissue) were seen as compared with matched controls (39.33µg/g tissue) the statistical analysis of the same was found to be not significant (as shown in Table 2) which could be attributed to the smaller sample size.

In a study by Sezer et al[6] in the serum involving 40 cases and 40 controls, a higher value of MDA in patients with Lichen planus with a mean value of 18.24µg/g tissue +/- 5.2µmol/L was obtained in comparison to that of the control group which is similar to the results obtained in the present study.

Studies done by Ines Dammak et al[15], and Rai Balwant et al[16] have also reported that higher levels of MDA were observed in the tissue of patients with lichen planus.

Rai Balwant et al.[16] demonstrated elevated levels of MDA in cases with periodontitis (p-value of < 0.05) and concluded that free radicals had a role in the pathogenesis of periodontitis.

Lipid peroxidation can alter cell signaling or act as toxic second messengers that amplify damage. Such by-products include 4- hydroxynonenal, Malondialdehyde etc which induce apoptosis. This could explain the reason for apoptosis in lichen planus.

In our study, we found significantly higher levels of SOD, and relatively higher levels of protein and MDA in the tissue samples of oral lichen planus contributing to the pathogenesis of lichen planus. Further research with larger sample size are required to conclusively prove the role of oxidative stress and lipid peroxidation in the pathogenesis of lichen planus.

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Conflict of Interest: Nil

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