Evaluation of oxidant stress in the patients of oral lichen planus using malondialdehyde

Altaf Hussain Chalkoo, Prenika Sharma, Nusrat Nazir and Zahoor Ahmed

Abstract

Introduction: Oxidative stress and antioxidant defense mechanism may play an important role in the pathology of lichen planus.

Objectives: Evaluate the levels of MDA in serum and saliva of patients with OLP and to compare the levels.

Methods: The study included 30 cases of OLP patients which formed the study group and 30 cases of healthy individuals, which formed the controls.

Results: The levels of MDA were significantly higher in serum and saliva of cases with lichen planus than in normal.

Conclusion: Salivary and serum MDA were found to be higher in oral lichen planus patients which suggested that saliva can be used as an alternate and effective diagnostic tool in evaluating the oxidative stress status of an individual.

Keywords: Malondialdehyde (MDA, thiobarbituric acid (TBA), free radical (FR))

1. Introduction

Lichen planus is a chronic inflammatory disease that affects the skin and the mucous membrane [1]. Oral lichen planus (OLP), the mucosal counterpart of cutaneous lichen planus, presents frequently in the fourth decade of life and affects women more than men in a ratio of 1.4:1. In the majority of patients there is no associated cutaneous lichen planus or lichen planus at other mucosal sites. This may be called “isolated” OLP [2]. The exact pathogenesis of LP is not yet understood. Cytokine-mediated apoptosis of basal keratinocytes as a result of the accumulation of activated T-cells in the dermoepidermal junction had been proposed as one mechanism, but the initial antigen that triggers this process has not yet been identified.

Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) The role and importance of oxidative stress has been suggested in the pathogenesis of LP.

Lipid peroxidation leads to the formation of Malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic [3]. MDA has also been implicated in the pathogenesis of several pathological disorders including OLP. As salivary secretions represent the oral microenvironment, estimation of salivary levels of MDA along with serum will reveal the oxidative stress status of OLP more effectively than with estimation in serum alone.

Estimation of oxidative stress markers in OLP will help unraveling the pathogenesis, which in turn help improve the therapeutic options in the treatment of OLP. The present study aims at estimation of MDA in serum and saliva samples of OLP and to compare their levels [4]. There is less data available in the literature regarding the oxidant stress status in patients of oral lichen planus. So, the present study was designed to evaluate the status of oxidative stress in patients of oral lichen planus belonging to ethnic Kashmiri population.
2. Materials and Methods

Study was performed in department of oral medicine and radiology, government dental college Srinagar. Patients were selected irrespective of age and gender. A written informed consent was obtained from all patients and control subjects. Study was approved by the internal Research Ethical Committee of our Institution. The present study included 30 cases of oral lichen planus. Most of the patients were fresh cases visiting outpatient department, and few were follow up cases. Cases were clinically and histopathologically diagnosed for oral lichen planus. Patient treated with immunosuppressive agents, steroids, NSAIDS, topical medications for the last 4 weeks, history of cigarette smoking, alcohol consumption, intake of drugs (vitamin E and vitamin C), and possible history of trauma or surgery during the last four weeks, systemic diseases, malignancies were excluded. The control subjects were 30 healthy individuals from the OPD, matched for age and gender. All serum and saliva samples were double blinded in order to avoid bias of values during estimation procedures. The subjects in the experimental groups were asked to rinse their mouth with water for 2 min and then the patients were asked to wait for a minute, after which unstimulated saliva, about 1 ml was collected in a sterile graduated test tube. In order to neutralize the pH, the sample was then diluted with 10 ml of phosphate buffered saline. After diluting, the mixture was centrifuged for 5 min at 3000 rpm and the supernatant fluid was stored at -80 °C until use. For collecting serum samples, 2ml venous blood was taken from patients and transferred to sterile test tubes. The blood was allowed to clot and was centrifuged for 5 min at 3000 rpm. The clear serum was separated and stored at -80 °C until analysis. For estimation of MDA, serum and saliva samples were obtained, and they were thawed to room temperature. 2.5 ml of trichloroacetic acid was mixed with 0.5 ml of sample (plasma and serum). The contents were mixed well and incubated for 15 min at 90 °C. The samples tubes were cooled with cold water. The contents were centrifuged at 3000 rpm for 10 min. 2 ml of supernatant was transferred to a new tube. To this 1 ml 0.675% thiobarbituric acid was added. The tubes were sealed and incubated at 90 °C for 15 min. And the content were measured at 586 nm using Microplate Reader. The average net optical density was calculated by subtracting the average value of sample (V1) from the average value of control (V0) and dividing with the average value of control (V0). Using the obtained absorbance value for each sample, the concentration of MDA in serum and saliva samples were determined. The P value between cases and control in serum and salivary NO and MDA in both patients and control. Values < 0.005 considered significant.

3. Result

Statistical analysis was performed by using statistical package for social sciences (SPSS VERSION 17) and inferences were drawn. Student’s t-test was used to determine the statistical significance of serum and salivary NO and MDA in both patients and control. Values < 0.005 considered significant.

### Demographic Characteristics of Study Population

<table>
<thead>
<tr>
<th>Clinical type of oral lichen planus</th>
<th>Number of Study Participants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular</td>
<td>21 (70.0%)</td>
</tr>
<tr>
<td>Erythematous</td>
<td>6 (20.0%)</td>
</tr>
<tr>
<td>Plaque</td>
<td>2 (6.6%)</td>
</tr>
<tr>
<td>Ulcerative</td>
<td>1 (3.3%)</td>
</tr>
</tbody>
</table>

### Clinical Varieties of Oral Lichen Planus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Mean In serum (μg/ml)</th>
<th>Mean In Saliva (μg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA Assay</td>
<td>Study Group</td>
<td>47.8</td>
<td>60.7</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control Group</td>
<td>21.0</td>
<td>23.4</td>
<td></td>
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</table>
4. Discussion and Conclusion
The role of oxidative stress in the etiopathogenesis of oral lichen has been described by some researchers through estimating the levels of oxidative markers like MDA in various samples—serum, saliva, and tissue. MDA has been presumed as the main production of unsaturated fatty acids' peroxidation which can lead to reduction in cellular membrane permeability, devastating cellular structure and function which is incorporated in pathogenesis of most of disease [5]. Reactive oxygen metabolites lead to destruction and damage to cell membranes by lipid peroxidation (Sertan et al. 2011)[6]. Increased membrane lipid peroxidation is considered to evoke immune and inflammatory responses, and to activate gene expression and cell proliferation. The inflammatory cellular infiltrate in LP, which consists mainly of CD4 + lymphocytes, is a well-known source of ROS. We believe that our findings show that there was a disturbance in the anti-oxidant defense mechanism leading to increased production of ROS, thus resulting in increased lipid peroxidation and its product MDA [7].

The origin of this cellular degeneration is believed to be attributed to sub epithelial infiltration of T-lymphocytes that contributes to the local production of cytokines. Cytokines can stimulate production of ROS. The presence of apoptosis is a hallmark feature in lichen planus and this indicates that ROS may play a crucial role in the disease process, as ROS are essential mediators of apoptosis. This oxidative damage to the tissues may be a result of lipid peroxidation [8].

In our study we found and increased level of serum as well as saliva Malondialdehyde in oral lichen planus patient compared with their controls. Similar study done by Sezer et al. showed an increased serum level of MDA in skin lichen planus subjects in comparison with healthy ones (P = 0.031). Also Agha-hosseini et al. research which observed a higher level of MDA in unstimulated whole saliva of patients with OLP than healthy subjects [9]. It is suggested that patients with OLP are more susceptible to an imbalance of antioxidant-oxidative stress situations.

Antioxidant elements protect cell membranes against lipid peroxidation by reducing free radicals and their subsequent oxidative damages. Saliva may constitute a first line of defense against FR-mediated oxidative stress, because the process of mastication promotes a variety of such reactions, including lipid peroxidation. The increased membrane lipid peroxidation is considered to evoke immune and inflammatory responses and to activate gene expression and cell proliferation [10]. Salivary levels of MDA were found to be significantly higher than serum levels. This shows that saliva can be used as an effective diagnostic tool in evaluating the oxidant stress of an individual. Monitoring the oxidative stress status of lichen planus can be used for therapeutic management. Antioxidants may prove to effective in treatment of lichen planus.

5. References
8. Ergun et al. Evaluation of oxidative stress and