Original Article

Evaluation of salivary oxidative stress in oral lichen planus using malonaldehyde

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Abstract

Background: Oral lichen planus is a chronic, mucocutaneous, inflammatory disease, with an unknown etiology. Reactive oxygen species and oxidative damage to the tissues might be the cause. Malonaldehyde (MDA), a low molecular weight end product of lipid peroxidation reaction is a suitable biomarker of endogenous DNA damage. monitoring the oxidant-antioxidant status of saliva may serve as an efficient marker of disease development in oral lichen planus patients.

Aim and Objectives: To evaluate salivary oxidative stress in oral lichen planus subject using MDA and compare it with control subjects. Furthermore, to compare MDA levels in erosive and hypertrophic lichen planus.

Materials and Methods: The current study is case–control study. Unstimulated salivary samples in the morning hours were taken from oral lichen planus subjects ($n = 25$) and controls subjects without any oral disease ($n = 25$). The saliva was centrifuged at 900 g for 10 min at a temperature of 4°C. Then, the entire filtrate was transferred to Eppendorf test tubes and frozen at–80°C until analysis. Salivary MDA was done through thiobarbituric acid reactive substance assay as per the protocol laid down by the manufacturer (Sigma Aldrich Lipid Peroxidation Assay Kit).

Results: The data were expressed as the mean ± standard deviation and the statistical analysis was done using Student's *t*-test using SPSS version 21 IBM software. The salivary level of MDA was significantly higher than that of controls ($P < 0.05$).

Conclusion: The higher level of MDA in patients with oral lichen planus suggests that free radicals and the resulting oxidative damage may be important in the pathogenesis of oral lichen planus lesions.

Keywords: Malonaldehyde, oral lichen planus, oxidative stress, potentially malignant lesion, saliva

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INTRODUCTION

Lichen planus is a chronic inflammatory condition that may affect the skin, scalp, nails, mucous membranes (especially mouth) and the genitalia.[1] Oral lichen planus is a chronic mucosal condition commonly encountered in clinical dental practice.[2] An overall age-standardized global prevalence

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is 1.25% (0.96% in men and 1.57% in women) in general population with a malignant transformation rate of 0.4%–1.74%[3]

In recent years, there has been an increasing research interest in the role of oxidative stress in pathogenesis of OLP. Oxidative stress is a disturbance in the equilibrium status of pro-oxidant and anti-oxidant reaction. Activated T-cell release cytokines leading to the attraction of inflammatory cells and the destruction of keratinocytes by cell-mediated cytotoxicity.[4] Cells such as keratinocytes, fibroblasts and inflammatory cells release reactive oxygen species (ROS) may inflict cellular damage by overwhelming the antioxidant defense mechanisms leading to excessive production of ROS.[5]

It is assumed that markers of oxidative stress are associated with different local oral condition. The level of antioxidant is a potential determinant of susceptibility to be affected by OLP. This suggests that oxidative stress is a major trigger for OLP.[5]

Saliva, as a body fluid in the oral cavity, contacts directly with the local environment. Saliva has numerous benefits, as it is easily accessible, painless and noninvasive, less time-consuming. Furthermore, it can be used for mass screening of large population samples.[6]

Only few studies have been published assessing oxidant-antioxidant status in patients with selected clinical forms of OLP in comparison with healthy individuals without symptoms of the disease.[7-9] The objective of the current study was to evaluate salivary oxidative stress in Oral Lichen Planus subjects using malonaldehyde (MDA) and compares it with control subjects and evaluate salivary oxidative stress using MDA in sub-groups of oral lichen planus.

MATERIALS AND METHODS

The current study was a prospective case-control study. Institutional ethical clearance and informed consent from the subject was duly obtained. The patient's demographic profile was noted, brief history was taken including a history of relevant risk factors and habits. Clinical examination was performed and type of lichen planus was recorded. Lichen planus was diagnosed as per the criteria put forth by the World Health Organization with slight modification[10] [Table 1 and Figure 1].

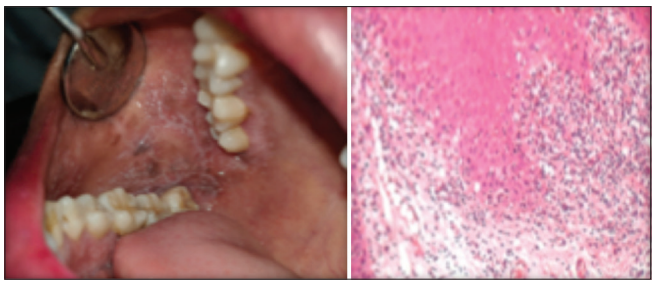

Figure 1: Clinical and histopathological picture (×40) of oral lichen planus (Case No: OLP-01)

Subjects were divided into two groups:

- Group A: Patients with Established cases of oral lichen planus (n = 25)
  - Subgroup A: Erosive oral lichen planus
  - Subgroup B: Reticular oral lichen planus
- Group B: Age- and sex-matched normal healthy subjects (n = 25) with no oral lesions and no habits.

Subjects with age above 18 years and below 50 years of age of either gender with no systemic illness and cases of control group having no morbidity were included and only established cases of oral lichen planus were included in subject group. Subjects having autoimmune disease or malignancy, history of trauma or surgery, on long-term steroid or other medications, smokers or having periodontitis or diabetes mellitus were excluded from the study.

Procedure

The subjects were asked to rinse their mouth with water before collecting specimen. Un-stimulated salivary samples were taken. The spitting method was used for saliva collection and the total time duration of saliva collection was approximately 5 min. All samples were collected between 8 AM and 11 AM to avoid diurnal variation.

Salivary samples were collected in the morning after 8 h of fasting. The sample was immediately placed into ice and then taken to laboratory. The saliva was centrifuged at 900 g for 10 min at a temperature of 4°C. Then the entire filtrate was transferred to sterile 1.5 ml micro test tubes and adding 15 µl solution of butylated hydroxytoluene (BHT) in ethanol per 1 ml of saliva so as to prevent lipid peroxidation during sample storage and frozen at −80°C until analysis. Salivary MDA was estimated through thiobarbituric acid reactive substance assay (TBARS assay) as per the protocol laid down by the manufacturer (Sigma Aldrich Lipid Peroxidation Assay Kit).

Thiobarbituaric acid (TBA) solution was prepared by reconstituting the given TBA solution with 7.5 mL glacial acetic acid and then adjusted the final volume to 25 mL with water. MDA Standards were prepared by diluting 10 µL of the 4.17 M MDA Standard Solution with 407 µL

Table 1: Modified WHO criteria for diagnosing oral lichen planus[10]

Clinical criteria	Histopathological criteria
Presence of bilateral, more or less symmetrical lesions Presence of a lacelike network of slightly raised grey-white lines (reticular pattern) Erosive, atrophic, bullous and plaque type lesions are accepted only as a subtype in the presence of reticular lesions elsewhere in the oral mucosa In all other lesions that resemble OLP but do not complete the above-mentioned criteria, the term "clinically compatible with" should be used Erosive lichen planus manifests as atrophic and erythematous areas frequently surrounded by thin striae	Presence of a well-defined band-like zone of cellular infiltration that is confined to the superficial part of the connective tissue, consisting mainly of lymphocytes Signs of liquefaction degeneration in the basal cell layer Absence of epithelial dysplasia When the histopathological features are less obvious, the term "histopathologically compatible with" should be used

OLP: Oral lichen planus

of water to prepare a 0.1 M MDA Standard Solution. We further diluted 20 μL of the 0.1 M MDA Standard Solution with 980 μL of water to prepare a 2 mM MDA standard. We added 0, 2, 4, 6, 8 and 10 μL of the 2 mM MDA standard solution into separate microcentrifuge tubes, generating 0 (blank), 4, 8, 12, 16 and 20 nmole standards. Water was added to each tube to bring it to the final volume.

Saliva samples were gently mixed with 42 mM sulfuric acid in a microcentrifuge tube. We added phosphotungstic acid solution to it and vortex the solution. The samples were incubated at room temperature for 5 min and then centrifuged the samples at 13,000 rpm for 3 min. In a separate tube, 2 μL of BHT (×100) was added. Then, we re-suspended the pellet on ice with the water-BHT solution and adjusted the volume with water. TBA solution was added to each vial containing standard and sample and incubated at 95°C for 60 min. It was then cooled down to room temperature in an ice bath for 10 min. The resultant solution was matched with the MDA standards and measured absorbance at 532 nm [Figure 2].

Statistical analysis

Statistical evaluation was done using analysis of variance for scale and ordinal variance. Independent Student's *t*-test was applied using SPSS version 17 Software (IBM corporation, NY, United States).

RESULTS

A total of 50 subjects participated in the study, of which 25 patients were from the study group (14 males and 11 females) while 25 subjects from the age- and sex-matched the control group (13 males and 12 females). The mean age of the control group was 44.4 ± 5.65 years and that of the control group was 43.12 ± 6.24 years [Figure 3]. In the study group, 11 cases were of erosive lichen planus (Subgroup A) while 14 cases were reticular lichen planus (Subgroup B).

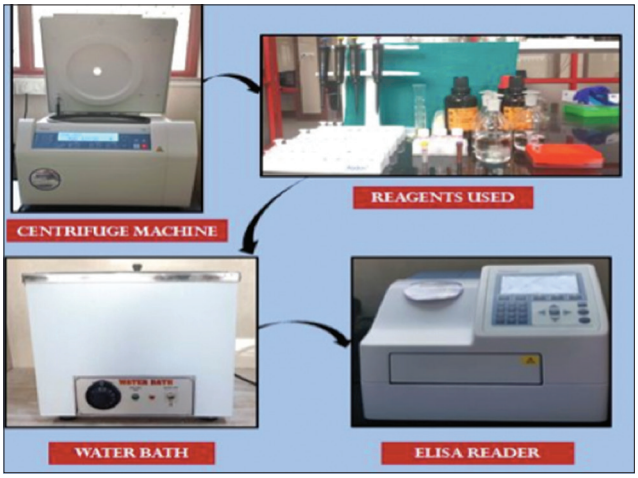

Figure 2: Reagents and equipments used in the study

The mean MDA value in the case group was 0.13226 ± 0.108027 nmol/L (mean ± standard deviation) with standard error (SE) of 0.021605. The mean MDA value in the control group was 0.06844 ± 0.040827 nmol/L (with SE of 0.008165. Our study showed higher salivary MDA in cases than in controls and the difference was statistically significant ($P < 0.01$) [Table 2].

In between subgroups, MDA value of subgroup A 0.400 ± 0.236 nmol/L (SE - 0.071) and in Subgroup B: 0.279 ± 0.221 nmol/L (SE - 0.059). This value was statistically nonsignificant ($P = 0.184$) [Table 3].

DISCUSSION

Lichen planus, first described by Erasmus Wilson in 1869, is a pruritic dermatosis of known etiology that affects the skin and mucous membranes.[11] The exact etiopathogenesis of OLP is unknown, but it is believed to result from an abnormal immune response in which basal epithelial cells are recognized as foreign because of changes in the antigenicity of their cell surface.[12]

The origin of this cellular degeneration can be accredited to subepithelial infiltration of T-lymphocytes that contributes to the local production of cytokines, which in turn can

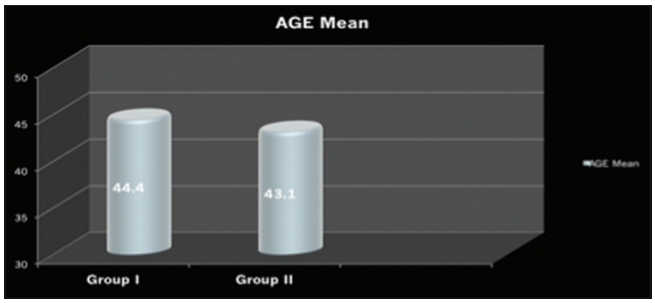

Figure 3: Mean age in case and control

Table 2: Comparison of mean between cases and control

Mean Values between cases and controls				
Group	***n***	**Mean±SD**	**SEM**	**Significant (two-tailed), *P***
Group I (case)	25	0.13226±0.108027	0.021605	0.008 (s)
Group II (control)	25	0.06844±0.040827	0.008165	

SD: Standard deviation, SEM: Standard error of mean

Table 3: Comparison of mean between two sub-groups

Comparison between different sub-group				
	n	**Mean±SD**	**SEM**	**Significant (two-tailed), *P*>0.05**
Subgroup A	11	0.400±0.236	0.071	0.184 (NS)
Subgroup B	14	0.279±0.221	0.059	

SD: Standard deviation, SEM: Standard error of mean, NS: Not significant

stimulate the production of ROS and cause oxidative damage to the tissues. Reactive oxygen metabolite leads to destruction and damage to cell membranes by lipid peroxidation.[5]

Oxidation of membrane-associated polyunsaturated fatty acids of phospholipids results in lipid peroxidation, which has been considered a foremost contributor of oxidative stress.[13] The end product of lipid peroxidation, MDA is a distinguished marker of free radical-mediated damage and oxidative stress.[14] Saliva is a more striking biological sample for clinical studies on oral diseases due to its diagnostic, prognostic and therapeutic responses.

Sezer *et al.* reported that there was increased oxidative stress and lipid peroxidation together with an imbalance in the antioxidant defense system in patients with cutaneous lichen planus, suggesting that ROS might be involved in the pathogenesis of lichen planus.[15] Sertan *et al.*, Sezer *et al.* obtained increased serum MDA levels in oral lichen planus.[15,16] According to Upadhyay *et al.*, serum levels of MDA were significantly higher in OLP and oral lichenoid reaction than in controls but there was no significant difference in serum MDA levels between oral lichen planus and oral lichenoid reaction patients.[17] Ergun *et al.* found a positive correlation in serum and salivary MDA levels in OLP patients.[18] Agha-Hosseini *et al.* found MDA level is higher in saliva samples of OLP cells in comparison to controls similar to our study.[19] Vlková *et al.* found twice TBARS level in premalignant lesions.[20] Abdolsamadi *et al.*, Rekha *et al.* and Abbas *et al.* also found increase in salivary MDA level in in OLP.[8,21,22] Mehdipour *et al.* found MDA levels in OLP and controls to be nonsignificant.[23] The probable reason for varying results could be different geographical location. Although studies have been done on estimation on salivary samples we found only one study, which compared oxidative stress level in two variants of oral lichen planus. The result of Darczuk *et al.* was inconsistent with our study.[9] The most probable reason being variability in sample size. Most of the studies comply with the finding of our study, that is, increased oxidative stress (MDA levels) in different biological media, i.e., serum, saliva and tissue.

Mishra and Maheshwari Uma *et al.* in their systematic review concluded that different studies suggest an increased oxidative stress and imbalance in the antioxidant defense system in biological fluids of patients with oral lichen planus, thus proving that oxidative stress plays an important role in its pathophysiology.[24]

Rai performed a clinical trial by treating oral lichen planus patients with curcumin and measure oxidative stress in serum and saliva using MDA and found that there is reduction in serum and salivary levels of MDA posttreatment after 7 days as well as after 209 days.[25] Increased oxidative stress and imbalance in the antioxidant defense system in biological fluids of patients with oral lichen planus, proved that oxidative stress plays an important role in its pathophysiology of oral lichen planus.

In our present study, the mean MDA levels in the control population were 0.06 nmol/L. In subjects with oral lichen planus, the mean MDA level was 0.13 nmol/L. Our findings implicate that there is an increase in MDA Levels from control to OLP subjects.

CONCLUSION

The oxidative stress has a role in etiopathogenesis of oral lichen planus. Although the mean MDA values were higher in erosive variant than reticular variant of OLP, there was no statistical difference between the two variant of oral lichen planus. Antioxidants need to be incorporated as an essential part of treatment protocol to overcome the antioxidant deficiency. Further studies with a large sample size are required for determining its usefulness.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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