



ANTIOXIDANT CAPACITY IN PATIENTS WITH ORAL LICHEN PLANUS AND HEALTHY SUBJECTS-A COMPARATIVE STUDY

Dental Science

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ABSTRACT

BACKGROUND AND AIM: Oral lichen planus is a common chronic inflammatory disease of the oral mucosa with malignant potential. There are various antioxidant systems in body fluid which can neutralize the reactive species. Impaired function of these systems leads to oxidative damage in the tissues and can result in the inappropriate activation of the immune system. Thus reduced antioxidant defense and increased oxidative stress may play an important role in etiology of many chronic immune mediated diseases such as: diabetes, psoriasis, vitiligo. Analyzing the antioxidant capacity in patients with OLP is of significant value as it can provide essential clues in the etiology of the diseases and help in better management of the disease. This study aimed to assess and compare the antioxidant capacity of oral lichen planus patients and healthy subjects.

MATERIALS AND METHODS: This study included 50 oral lichen planus patients and 50 healthy subjects.

DPPH Assay is used to assess the antioxidant capacity in OLP patients and healthy subjects.

The percentage scavenging of DPPH radical gave the antioxidant activity of the sample.

RESULTS: Antioxidant level were reduced in the OLP group while comparing it with the control group.

Mean antioxidant level in OLP group was 0.6 and that of the control group was 1.6. Comparison of mean antioxidant levels between the OLP group and control group showed significant ($p < 0.0001$) difference. The difference between the reticular and erosive OLP group showed significant difference with p value less than 0.0001.

CONCLUSION: Antioxidant level significantly decreased in OLP group compared to control group. Erosive OLP patients show low antioxidant level compared to reticular OLP patients. The findings of present study support the hypothesis of the association between antioxidant capacity and OLP.

KEYWORDS

Antioxidant status, Oral lichen planus, Total antioxidant capacity

INTRODUCTION

Lichen planus (LP) is a chronic immunologically mediated mucocutaneous disorder.¹ It affects all irrespective of age, sex and geographical location. Oral lichen planus is bilateral in presentation. They can be lacy or reticular, annular, patches or strings.

The characteristic feature of OLP is the Wickham's striae, which is found on the surface of the lesion, formed by very fine grayish white lines.² The clinical evaluation of the oral lesions is based on the six clinical forms described by Andreason: reticular, papular, plaque, atrophic, erosive and bullous.³ Burning sensation and sometimes pain usually accompany the erosive, atrophic or bullous type lesion. The reticular form has a better prognosis as 40% of cases has spontaneous remission, the erosive type being long standing and with frequent exacerbations and severe pain and complications.² Periods of remission are seen in oral lichen planus where the symptoms and lesion appear and regress at intervals.

The newest diagnostic approach for OLP lesions was published in 2016 by American Academy of Oral and Maxillofacial Pathology.⁴

CLINICAL CRITERIA:

Multifocal symmetric distribution white and red lesions exhibiting one or more of the following forms:

- reticular/papular
- atrophic (erythematous)
- erosive (ulcerative)
- plaque
- bullous

lesions are not exclusively localized to the sites of smokeless tobacco placement adjacent to and in contact with dental restorations lesion onset does not correlate with the start of a medicine use of cinnamon-containing products.

Histopathologic Criteria:

Band-like or patchy, predominately lymphocytic infiltrate in the lamina propria confined to the epithelium- lamina propria interface basal cell liquefactive (hydropic) degeneration lymphocytic exocytosis absence of epithelial dysplasia absence of verrucous epithelial architectural change.

Etiology of OLP is unknown. Multiple precipitating factors of OLP are psychological stress, anxiety, diabetes mellitus, hypertension, autoimmune diseases such as alopecia areata, dermatitis herpetiformis, myasthenia gravis etc.⁵ Oxidative stress is one more important precipitating factor in the progression of lichen planus.

Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and the ability of the biological system to readily detoxify the reactive intermediates or easily repair the resulting damage.

ROS production basically relies on enzymatic and nonenzymatic reactions. Enzymatic reactions that are able to generate ROS are those involved in respiratory chain, prostaglandin synthesis, phagocytosis, and cytochrome P450 system.^{6,7} Nonenzymatic reactions can also be responsible for free radical production, that is, when oxygen reacts with organic compounds or when cells are exposed to ionizing radiations. Nonenzymatic free radical production can also occur due to mitochondrial respiration.⁸

ROS are mainly produced by mitochondria, during both physiological and pathological conditions and formed by cellular respiration, by lipoxygenases (LOX) and cyclooxygenases (COX) during the arachidonic acid metabolism, and by endothelial and inflammatory cells. When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids.

Excessive ROS which is not neutralized by antioxidant systems can react with DNA bases and lead to mutation which can mediate carcinogenesis. Excessive ROS can also lead to sustained pro-inflammatory cytokine activation. In OLP, excessive ROS can be a possible factor which can increase severity of the disease, prolong healing periods and also lead to malignant transformation.

Antioxidants act as free radical scavengers and neutralize excess of reactive oxygen species (ROS). Anti-oxidant system consist of (1) enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and thioredoxin (Trx) as well as the non-enzymatic antioxidants. The enzymatic antioxidants can directly or indirectly neutralize the ROS to protect the cells. Non-

enzymatic antioxidants can neutralize the oxidative effect by promotion of anti-oxidative enzyme or directly processing oxidative chain reaction. The most important minerals which have the antioxidant function are Selenium and Zinc. When there is breakdown of antioxidant pathways, there will be overexposure to certain ROS which leads to oxidative stress. Vitamins such as tocopherols (vitamin E) and ascorbic acid (vitamin C) as well as the carotenoids react with free radicals, notably peroxy radicals, and with singlet molecular oxygen, this being their basis of function as antioxidants.

Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution, being used to evaluate the antioxidant capacity of biological samples.¹⁰ The measurement of the total serum antioxidant capacity represent a suitable biochemical parameter for evaluating the overall antioxidant status resulting from antioxidant intake or production and their consumption by the increasing levels of oxidative stress.¹¹ Antioxidant capacity is related with compounds capable of protecting a biological system against the potentially harmful effect of processes or reactions involving reactive oxygen and nitrogen species (ROS and RNS).¹²

Total antioxidant capacity (TAC) can be a good marker for evaluation of patients with oral lichen planus. It may detect a possible synergism between known antioxidants and the contribution of unknown or rarely estimated antioxidants.¹³ In OLP patients, total antioxidant defense was significantly lower than that in healthy subjects in their serum samples.¹⁴

Lower levels of Total Antioxidant Capacity (TAC) in OLP patients compared to the controls, indicates decreased antioxidant activity due to oxidative stress. Reduced antioxidant capacity in OLP may result in increased oxidative process-mediated cellular damage.¹⁵

Measuring total antioxidant capacity in body fluids may serve as a diagnostic biomarker and as an innovative tool to assess the disease progression or resolution during follow up of this chronic disease as measures complementary to clinical monitoring.

- The aim of the study was to assess and compare the antioxidant capacity between oral lichen planus patients and healthy subjects.

MATERIALS AND METHODS

Patients reported to the department of oral medicine and radiology, Kannur dental college.

For the case group, the following inclusion and exclusion criteria were considered.

INCLUSION CRITERIA

1. Patients diagnosed with Oral lichen planus.

EXCLUSION CRITERIA

1. Patients who are under psychiatric treatment
2. Pregnant patients.
3. Patients who are under long term antioxidant therapy.

METHODOLOGY

A written consent was obtained from all subjects participated in the study prior to the study procedure.

Group 1- consisted of 50 age and sex matched subjects without any signs of oral lesions.

Group 2- included patients with diagnosed Oral lichen planus based on the modified WHO criteria for OLP diagnosis.

After obtaining the informed consent from each subjects, a detailed case history was recorded in all the subjects. Grading of OLP was done based on the Pinboonniyom et al grading system and pain assessment was done using Visual analogue scale (VAS) scale.

Blood sample is collected from all patients involved in the study in a clot activator tube.

Blood sample is centrifuged and serum separated from all blood samples and transferred to Eppendorf tube and stored in -4° celsius in a deep freezer.

Estimation of total antioxidant capacity of human blood serum DPPH radical scavenging assay: (1,1 -diphenyl -2-picrylhydrazyl solution) Using UV-Visible light Spectrophotometer

DPPH assay method is very simple and is also quick for manual analysis of antioxidant contents. The DPPH test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors.

About 4.3 mg of DPPH was dissolved in 3.3 ml methanol and protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3 ml methanol, and the absorbance was taken immediately at 517 nm for control reading. 50 µl of various concentrations of compounds as well as standard compound (e.g., human serum albumin) were taken, and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3 ml and to each 150 µl DPPH was added. 150 µl of freshly prepared DPPH solution in methanol was added to 50 µl of centrifuged blood serum.

The samples were kept in darkness for 30 min at room temperature and the absorption was then measured at 517 nm (Ac) against methanol as blank. The absorbance of the methanol solution of DPPH was also measured as control (Ac).

The absorbance was taken after 15 minutes at 517 nm using methanol as blank on UV-visible spectrophotometer.

The free radical scavenging activity was calculated using the following formula.

DPPH radical scavenging activity (%) = $\frac{[Ac - As]}{Ac} \times 100$. Ac – absorbance of control As – absorbance of sample

RESULTS

Comparison Of Mean Antioxidant Level Between Olp And Control Group

- Mean antioxidant level in control group was 1.6 and in OLP group was 0.60 with a mean difference of 0.99 and p-value of 0.0001 which is statistically significant. Significant decrease in antioxidant level was observed in OLP group compared to control group (Table 1 and graph 1).

Comparison Of Mean Antioxidant Level Between Reticular And Erosive OLP Groups

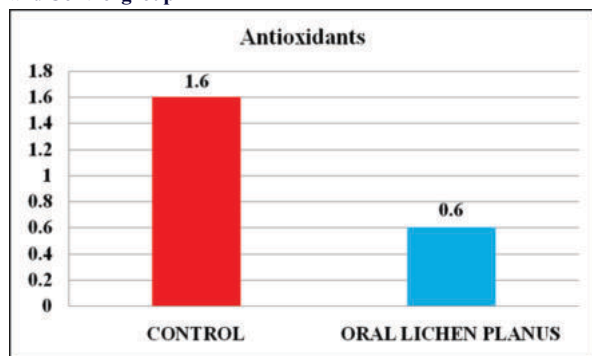
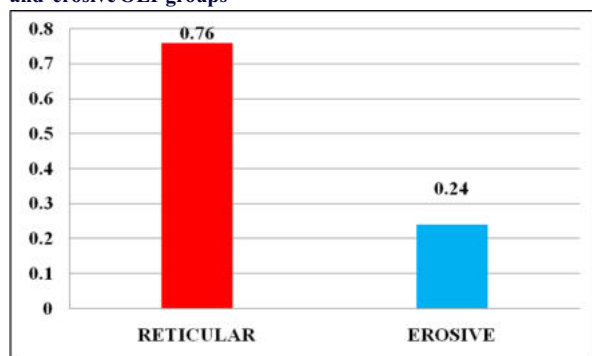
- Erosive OLP patients showed low antioxidant levels (0.24) compared to reticular OLP patients (0.76). The difference between reticular and erosive OLP group showed significant difference with a p-value of 0.0001 which is statistically significant (Table 2 and graph 2).

Table-1: Comparison of mean antioxidant level between OLP and Control group

Antioxidant	Group-I (n=50)	Group-II (n=50)	t	df	p value	Mean difference	95% confidence interval of the difference	
							Lower	Upper
Mean	1.60	0.60*	12.37	98	0.0001	0.99	0.83	1.15
Standard deviation	0.48	0.28						

Table-2: Comparison of mean antioxidant level between the reticular and erosive OLP group

Groups	N	Mean	Std. Deviation	t	df	P value	Mean difference	95% confidence interval of the difference	
								Lower	Upper
Reticular	35	0.76	0.16	10.59	48	0.0001	0.52	0.42	0.61
Erosive	15	0.24	0.13						
Total	50	0.33	0.15						

Graph-1: Comparison of mean antioxidant level between OLP and Control group**Graph-2: Comparison of mean antioxidant level between reticular and erosive OLP groups**

DISCUSSION

Reactive oxygen species play important role in physiological and immuno inflammatory reactions. In the human body, there is an antioxidant mechanism to maintain the balance of oxidation–reduction. The breakdown of this balance (i.e. increased ROS) could lead to increased damage directly by ROS. Indeed, several diseases have been correlated to an imbalance of oxidation–reduction or oxidative stress. There are two possible causes for the imbalance of oxidative stress: increased ROS with no concomitant or less increased antioxidant, or decreased antioxidant with no marked change of ROS.¹⁶ Total Antioxidant Capacity (TAC) can be a good marker for evaluation of oxidative stress in oral lichen planus patients. Increase in oxidative stress and imbalance in antioxidant defense system may be involved in the pathogenesis of oral lichen planus.¹³ In our study, percentage of mean antioxidant level in control group was 1.60 and in OLP group was 0.60. Significant decrease in antioxidant level was observed in OLP group compared to control group. This was in accordance with other studies in literature.^{17,18,19} Percentage of mean antioxidant level in reticular OLP group was 0.76 and in erosive OLP group was 0.24, which shows significant decrease in antioxidant level in erosive OLP group compared to reticular OLP group included in the study. This shows that antioxidant capacity decreases with severity of disease, i.e. severity of disease is inversely proportional with antioxidant level. Similar results observed in other studies in literature.^{20,21}

CONCLUSION

Total antioxidant capacity in whole blood of patients with oral lichen planus was significantly decreased as compared with healthy subjects. There is significant decrease in antioxidant capacity in erosive OLP group compared to reticular OLP group. It can be concluded that there is positive correlation with severity of disease and antioxidant capacity in oral lichen planus patients. When these results are extrapolated to clinical scenario, it can be seen that antioxidant supplementation should form an important part of treatment planning for management of OLP. Further studies on a larger sample size should be done for more evidence.

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