

A comparative evaluation of whole blood total antioxidant capacity using nitroblue tetrazolium reduction test in patients with oral lichen planus and healthy subjects

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Abstract

Background and Objectives: Oral Lichen Planus is an inflammatory disease of unknown etiology. Recently, reactive oxygen species have been implicated in the pathogenesis of this disease. The investigation of disease associated oxidant-antioxidant imbalance is hard because constrained availability of specific biomarkers of oxidative stress, and the fact that measurement of individual antioxidant may give a deceptive image due to the fact that antioxidants work in concert through chain breaking reactions. Therefore, analysis of total antioxidant capacity may be the maximum applicable investigation. As blood is continuously exposed to oxidative stress, the aim of this study was to analyze overall blood antioxidant potential in healthy and oral lichen planus patients through the use of Nitroblue Tetrazolium reduction test.

Methods: The study included 30 individuals which were selected from outpatients department with age ranging between 30 and 60 years, 15 patients in oral lichen planus group and 15 in healthy group (control), respectively. Blood samples were collected early morning and tested using Nitroblue Tetrazolium Reduction test (NBT). The test was performed and gave us the clear picture of whole blood total antioxidant capacity.

Results: Results were obtained, analyzed and results showed that the total antioxidant capacity in whole blood of patients with oral lichen planus was significantly decreased as compared with healthy group ($P < 0.001$).

Interpretation and conclusion: The decreased antioxidant capacity of total blood in oral lichen planus is indicative of the important role played by oxidative stress within the etiopathogenesis of oral lichen planus and warrants in addition investigation as it may offer a connection between chronic inflammatory diseases like oral lichen planus and plenty of other sicknesses that may be related to free radical formation.

Keywords: Lichen Planus, Oxidative Stress, Antioxidants, Reactive oxygen species (ROS).

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Introduction

Lichen planus is a chronic, inflammatory, papulosquamous disorder that may affect the skin, mucous membranes, genitalia, hair and nails.^[1] The exact pathogenesis is unknown, but lymphocyte and neutrophil functions are impaired in patients with oral lichen planus.^[2] Recently, increased reactive oxygen species and lipid peroxidation have been implicated to have a role in the pathogenesis of oral lichen planus.^[3,4] Studies have shown that mitogen-activated lymphocytes show increased nitroblue tetrazolium reduction.^[5]

According to the study by Aly and Shahin,^[6] the serum levels of NO were higher in patients with lichen planus than in the control group. NO and MDA are used as biomarkers for estimation of oxidative stress.

However, these studies concentrated on determining oxidative stress by acting on specific

biomarkers. A study conducted by Zilinskas J et al.^[7] put forward the fact that since antioxidants work through a chain breaking system, measuring these different antioxidants may give a deceptive picture of oxidative stress. The study conducted by Konuganti et al., which was based on this fact, demonstrated that the total blood antioxidant capacity of periodontitis patients was significantly lower (< 0.05) than the age and sex matched healthy controls using nitroblue tetrazolium reduction test.^[8]

Since the etiology of lichen planus is still unidentified, the treatment of the disease would be a symptomatic approach. Most of the previous studies in this field have been carried out on dermal and genital lichen planus subjects; ergo, the present study has been carried out to assess and evaluate the role of oxidative stress and antioxidant defense systems in patients with OLP by measuring total antioxidant activity levels and comparing them with normal subjects (controls).

Material and Methods

Patient Selection: Thirty patients attending routine dental examination at department of Oral Medicine and Radiology were recruited to the study. The age range of the patients ranged between 30 and 60 years, with fifteen patients in each: control and experimental groups. Controls subjects were age and sex matched. Written informed consent was obtained from all

patients and control subjects.

Diagnosis of oral lichen planus was done by clinical examination and signs and symptoms patients gave. A typical clinical presentation usually is a epithelial thickening arranged in a network pattern (Wickham's striae) with erythema surrounding mucosa, differentiating it from other mucosal lesions.

Inclusion criteria:

1. Patients with clinically diagnosed oral lichen planus.

Exclusion criteria:

1. Patients who were not willing to participate in the study.
2. Patients who had received any systemic steroids or other immunosuppressive drugs.
3. Patients with a history of trauma or any surgery one month prior to sampling.
4. Patients on chronic NSAIDs therapy.
5. Patients who were smokers.
6. Patients with lichenoid reaction.

Fasting venous blood samples (2 ml) were obtained from patients with oral lichen planus and healthy controls and were drawn into vacutainers containing heparin as an anticoagulant.

This study helped in the estimation of the whole blood total antioxidant capacity by using the reduction of Nitroblue Tetrazolium (NBT) method in patients with oral lichen planus and healthy controls. The aforesaid test was carried out in the Central Clinical Laboratory unit and followed in accordance with the ethical standards laid down by the University.

Preparation of NBT (Laboratory studies): NBT was obtained from Magnum Enterprises, Pune. (Dealers in Pathology and Cardiology Consumables, C-2, Pushkar Apartment, Near Om supermarket, Gokhale road, Pune

– 411016.

- a. 3mg of NBT was dissolved in 300 ml of normal saline (pH of 7.2).
- b. 1 ml of [A] was taken and diluted in 19 ml of normal saline with final pH of 7.2.

The final concentration of the prepared NBT is 1×10^{-4} mg/ml.

Procedure

Heparinized blood samples are incubated with buffered solution of freshly prepared NBT solution. Early morning blood samples or blood samples which were not collected more than 2 hours before the test were taken. In a cleaned test tube the heparinized blood was mixed with 2-3 drops of NBT solution, incubated and stored at 37°C for 15min. The supernatant layer was removed carefully by a pipet or centrifuged at 1000r/m for 3min. This layer was then taken and absorbance of NBT was measured in the samples by using spectrophotometer (wavelength of 580 nm).

Results

The institution facts: Samples were collected, test was performed and results were statistically analyzed using a 't' test and evaluated with 95% confidence interval.

Table 1: Showed mean and standard deviation (SD) of antioxidant levels which was 0.142mg/dl for healthy group and 0.048 in mg/dl for experimental group in whole blood.

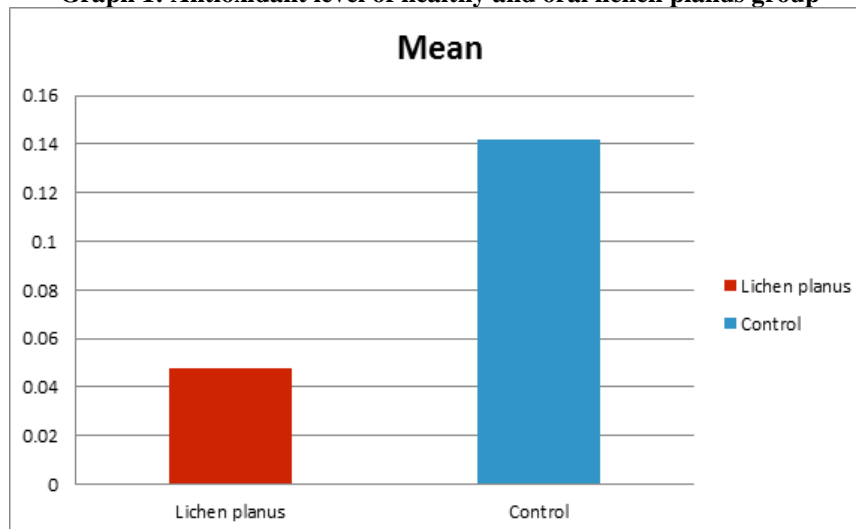
The student 't' test [Table 2] turned into used for statistical evaluation with 95% of confidence interval which confirmed that overall antioxidant capacity (TAOC) become substantially decrease in blood of oral lichen planus group patients [Graph 1] whilst in comparison with healthful group ($P < 0.001$).

Table 1: Group statistics

	Group	N	Mean	S.D
Anti-oxidant level (µg/ml)	Lichen planus	15	0.048	0.006
	Control	15	0.142	0.031
Anti-oxidant level (µg/ml)				
Antioxidant level (µg/ml)				

Table 2: Independent samples test

	t	df	P value	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Antioxidant level (µg/ml)	-11.320	28	<0.001*	-0.0941733	-0.1112138	-0.0771329

Graph 1: Antioxidant level of healthy and oral lichen planus group

Discussion

Oxidative stress is nothing but an imbalance between the production of free radicals and ability of the body to counteract their harmful effects. These harmful effects are neutralized by antioxidants, hence they play important role in measuring oxidative stress.

Lichen planus is an immunological mediated chronic disease, cause of which is still unknown. Many etiology were proposed out of which recently increased reactive oxygen species (ROS) and lipid peroxides thought to play important role.

Aim of our study was to evaluate the oxidative stress by measuring the whole blood antioxidant capacity in lichen planus patients and the results showed that patients with oral lichen planus have much lowered blood antioxidant capacity than healthy individuals.

This gives a clinical implication of including antioxidants therapy in treatment of oral lichen planus.

According to the study by Ergun et al.^[13], in OLP patients, salivary total antioxidant defense (TAA) was significantly lower than that in healthy subjects in their serum samples ($P = 0.01$). These findings, although measured in saliva, are in accord with the results of our study. Furthermore, the study by Shirzad et al.^[14] showed lower levels of salivary total antioxidant capacity in OLP patients compared to the controls, indicating decreased antioxidant activity due to oxidative stress. On the other hand, total antioxidant capacity level was higher in OLP patients compared to the controls according to the study by Agha-Hosseini et al.^[15] They suggested that such a discrepancy can be attributed to two possible reasons in the process of oxidative stress; first, the increase in ROS is observed with or without an increase in antioxidant system; second, antioxidant reduction occurs without any significant alteration in ROS.

Presently, no one method of all that have tried to measure the blood antioxidant levels can be

standardized because all these methods hire different biomarkers, different measurement indices and biological samples.^[10]

In addition biomarkers of ROS activity have very short half-life in vivo and cannot be measured directly.^[12]

One of the glitches with this study was the investigation of only reticular and erosive type of OLP. Larger sample size and other diagnostic modalities in combination may offer a better understanding of the disease process.

Conclusion

To conclude, our study showed a lower antioxidant capacity of whole blood in patients with oral lichen planus as compared to controls. Hence it proves oxidative insult has a vital role to play in etiopathogenesis of oral lichen planus. So we suggest adding antioxidant therapy should be a part of treating oral lichen planus cases.

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