

A study on role of oxidative stress and antioxidants in etiopathogenesis of Lichen Planus: A tertiary care hospital experience

Mithlesh Kumari¹, Naveen Kumar Singh², Priya Kaushik¹,
Sujata Sinha³, Arpita Suri⁴

ABSTRACT

Introduction: Lichen planus is an autoimmune mucocutaneous disease that primarily affects the mouth, skin, genital mucosa, scalp, and nails. In lichen planus, reactive oxygen species (ROS), increased oxidative stress, and an imbalance in antioxidant defence mechanisms may play a role in pathogenesis, posing a danger to disease prognosis. **Methods:** This was a case-control study conducted in a hospital. Forty newly diagnosed lichen planus patients over the age of 18 years who attended Dermatology & Venereology OPD served as cases, whereas forty healthy age and gender matched healthy volunteers served as controls. After discussing the goal and contents of the study, all participants in both groups signed a written and informed consent form. Serum MDA and SOD levels were determined using commercially available ELISA kits Human Malondialdehyde (MDA) ELISA kit (My biosource) and Human SOD ELISA kit (My biosource), respectively, and serum Uric Acid was calculated using commercially available ERBA kits (Uricase Peroxidase) based on standard photometric methods in the auto analyzer ERBA-XL (EM-200). **Results:** When lichen planus patients were compared to controls, the amount of oxidative stress marker serum malondialdehyde (MDA) was found to be significantly higher ($p < 0.0001$). Similarly, patients with lichen planus had significantly higher levels of antioxidant components, such as serum superoxide dismutase (SOD), while serum uric acid was statistically lower ($p < 0.0001$) compared to controls. **Conclusions:** Recent evidence has clarified that ROS generation activates both physiologic and pathologic mechanisms that are not specific to the skin but should be considered as general responses to pro-oxidant stimuli, suggesting that oxidative stress and antioxidant imbalance may play a key role in the pathogenesis of skin diseases. Increased oxidative stress and an imbalance in the antioxidant defence mechanisms in lichen planus may also play a role in the pathogenesis of lichen planus.

^{1,2,4} Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana

³ Department of Health Research, IRCS, New Delhi

Corresponding Author: Dr. Arpita Suri, Assistant Professor, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana, India **e-mail:** arpita.lhmc@gmail.com

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Introduction

Lichen planus (LP) is a subacute and chronic inflammatory dermatosis of the mucocutaneous surfaces that affects the skin, hair, nails; and mucous membranes and has a variety of clinical symptoms and histological findings [1-3]. The prevalence of lichen planus is unknown; however, it has been quoted to affect 1.5% of the total cases of skin diseases attending OPD in a tertiary care hospital [4]. In a study from India, 11.2% of cases were seen in children [5] while another study from Mexico showed this to be 10.2% [6]. Increased oxidative stress and lipid peroxidation, as well as an imbalance

in the antioxidant defence system, could promote oxidative stress in the development of lichen planus. The fluidity and signalling effectiveness of lipid-rich cell membranes are altered by peroxidation, resulting in inflammatory alterations and abnormal cell proliferation responses [7].

Malondialdehyde (MDA) is a byproduct of lipid peroxidation and one of the most common oxidative stress products [8]. Antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants including melatonin, uric acid (UA), and vitamins A and E, can protect against oxidative stress [9]. SOD is a

family of metalloenzymes, often known as antioxidant enzymes, that serve as the first line of defence against ROS-mediated harm. They catalyse the dismutation of superoxide anion free radical (O₂⁻) into molecular oxygen and hydrogen peroxide (H₂O₂), lowering the level of O₂⁻, which can harm cells at high levels. An imbalance in antioxidant status may cause H₂O₂ to build up, resulting in the vacuolization of the basal layer seen in LP [10]. Uric acid (UA) is a powerful antioxidant and scavenger of reactive oxygen species (ROS) and peroxynitrite. One of the main antioxidants in blood, UA, is a powerful free radical scavenger that also acts as a chelator of metal ions such as iron and copper by converting them to less reactive forms that cannot catalyse free-radical processes. Uric acid is also particularly effective at preventing peroxynitrite from nitrating the tyrosine residues of proteins, preventing cellular enzyme deactivation and cytoskeleton alteration [11]. An imbalance in the antioxidant defence system, as well as increased oxidative stress and lipid peroxidation, may play a role in the pathogenesis of LP [10]. Thus, serum levels of UA, MDA, and SOD were measured to assess the status of oxidative stress, antioxidant defence mechanisms, and inflammation in lichen planus patients from North India (Delhi-NCR). Oxidative stress also worsens the immunological process in lichen planus, bolstering the involvement of oxidative stress in the course of lichen planus pathogenesis.

Material and Methods

From October, 2019 to April, 2020, this hospital-based case-control study comprised newly diagnosed Lichen Planus patients over the age of 18 years who visited Dermatology and Venereology (OPD) of the hospital.

Patients with AIDS, Tuberculosis, history of smoking and/or drinking, hepatic or renal disease, rheumatoid arthritis, pregnant women, and patients on any prior treatment/therapy were excluded from this study.

As controls, 40 age and gender matched healthy volunteers from the general population were used. The Institutional Ethics Committee provided ethical approval. Both cases and controls signed a written informed consent form.

Outcome Variables

After an overnight fast, 5ml of venous blood was drawn from each individual in a simple vial, with all precautions observed. Serum was separated by centrifugation for 15 minutes at 3000 rpm and stored at -20° C for measurement of serum MDA and SOD. The amount of uric acid in the blood was checked right away.

Methods

Serum MDA levels

Commercially accessible ELISA kits Human Malondialdehyde (MDA) ELISA kit was used to test them (My biosource). The double-sandwich ELISA technique is used in the Human Malondialdehyde (MDA) ELISA kit. The pre-coated antibody is a human MDA monoclonal antibody, whereas the detecting antibody is a biotin-labeled polyclonal antibody. ELISA plate wells were filled with samples and biotin labelling antibody, then rinsed off with PBS (phosphate buffered saline) or TBS (tris-HCl buffered saline). Then, in order, Avidin-peroxidase conjugates were applied to ELISA wells; colouring was done with TMB (tetramethyl benzidine) substrate after the reactant had been properly washed away with PBS or TBS. TMB becomes blue when it is catalysed by peroxidase and then turns yellow when it is exposed to acid. The colour depth of the samples and the testing parameters were found to be positively associated.

Assay Procedure

1. The number of coated wells in the holder was set to the desired number, and a blank well was placed aside.
2. Samples or various concentrations of human MDA standard samples were introduced to corresponding wells (100 microlitre for each well), with the 0nmol/ml well filled with standard diluent. Seal the reaction wells with adhesive tapes and incubate for 90 minutes at 37°C.
3. The biotinylated human MDA antibody liquid was made 30 minutes ahead of time.
4. The Elisa plate was washed twice.
5. Each well was filled with 100 microlitre of biotinylated human MDA antibody liquid. Seal reaction wells with adhesive tapes and incubate for 60 minutes at 37°C.
6. The enzyme-conjugate liquid was made 30 minutes ahead of time.
7. Elisa plate was washed thrice.
8. Each well received 100 microlitre of enzyme-conjugate liquid, with the exception of the blank wells. Seal the reaction wells with adhesive tapes and incubate for 30 minutes at 37°C.
9. The Elisa plate was washed five times.
10. A 100 microlitre colour reagent liquid was applied to each individual well (as well as the blank well), and the eggs were laid in a dark incubator at 37°C. The hatching was stopped when the colour for the high concentration of the standard curve became darker and a colour gradient developed. Within 30 minutes, the chromogenic process should be under control.

11. Each well received 100 microlitre of Color Reagent C. (Also, into blank well). Mix thoroughly. Within 10 minutes, the OD was measured at 450 nm

Calculation of Results

The standard curve was hand-drawn. The concentration value of the samples was used as the abscissa, while the OD measurements were used as the vertical coordinate. A smooth line was employed to connect each coordinate point of the standard sample. Checking sample OD readings revealed the concentration of the samples. The sample was re-diluted and the experiment was redone when the sample OD exceeded the upper limit of the

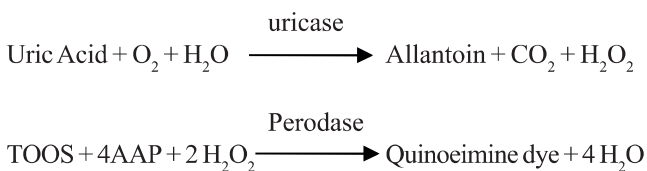
standard curve. When calculating the unknown, the result was multiplied by the dilution factor.

Serum SOD

The levels were calculated using a Human SOD ELISA kit (My biosource) in the same way that serum MDA was calculated.

Uric Acid in Serum

The estimation was done by commercially available ERBA kits (Uricase Peroxidase method) based on standard photometric methods in auto analyzer ERBA-XL (EM-200). The uric acid reagent is based on the principle of Trinder reaction. The series of reactions are involved in this assay system which is depicted as follows:



1. Uric Acid is oxidized to allantoin by uricase with the production of H_2O_2
2. The peroxide reacts with 4-aminoantipyrine (4-AAP) and TOOS in the presence of peroxidase to yield a quinoneimine dye. The absorbance was taken at 546 nm which is proportional to uric acid concentration in the sample.

Statistical Analysis

For sample size calculation we used prevalence as 1% and using prevalence formula we calculated the sample size as 16

at 95% confidence interval and 0.05 precision. We took a convenient sample size of 40 cases and 40 controls.

The data was entered into a spreadsheet, and the statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 software.

Means and standard deviations were used to summarise continuous variables. Bar diagrams were used to convey graphical data. The serum MDA, SOD, and uric acid levels of individuals with lichen planus and healthy controls were compared using the Student's independent t-test. The correlations between the parameters were determined using Pearson's correlation coefficient. For both parameters, the p-value ($p < 0.05$) was declared statistically significant.

Results

The mean age of lichen planus patients and controls were 34.17 ± 8.62 and 31.97 ± 7.69 respectively (Table-1). Twenty-three (57.50%) were males and 17 (42.50%) were females. In the control group, there were an equal number of males and females.

Oxidative Stress and Antioxidant status

When lichen planus patients were compared to controls, the amount of oxidative stress marker serum malondialdehyde (MDA) and antioxidant factor SOD were found to be significantly higher ($p < 0.0001$), as shown in Table1, although serum uric acid levels were statistically significantly lower ($p < 0.0001$) as compared to controls.

Association of serum MDA with anti-oxidant markers

The study found a statistically significant positive link between serum malondialdehyde (MDA) levels and superoxide dismutase (SOD) levels ($r = 0.69$, $p < 0.0001$), (Fig. 1) but a statistically significant negative correlation between malondialdehyde (MDA) levels and uric acid (UA) levels ($r = -0.63$, $p < 0.0001$).

Discussion

Lichen planus is a chronic autoimmune mucocutaneous disease that primarily affects middle-aged oral mucosa, skin, vaginal mucosa, scalp, and nails. According to reports, reactive oxygen species (ROS) and lipid peroxides play a role in lichen planus pathogenesis. Lichen planus (LP) [11] has

Table 1: Comparison of oxidative stress markers and antioxidant in lichen planus patients and controls.

Parameters	Cases (Mean \pm SD)	Controls (Mean \pm SD)	p-value
MDA (nmol/mL)	6.70 \pm 1.17	2.80 \pm 1.15	0.0001**
SOD (ng/mL)	8.65 \pm 2.14	3.50 \pm 1.33	0.0001**
Uric Acid (mg/dL)	3.48 \pm 0.87	5.80 \pm 1.13	0.0001**

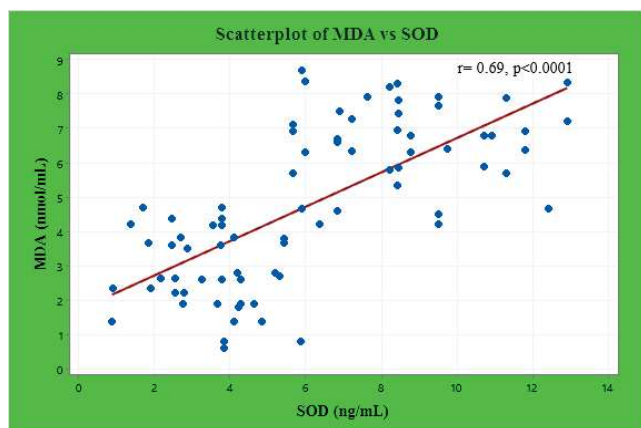


Figure 1: Graph depicting Pearson's correlation coefficient of serum malondialdehyde (MDA) levels with superoxide dismutase (SOD) of patients with lichen planus.

been linked to cardiovascular risks such as dyslipidemia, diabetes mellitus, and elevated oxidative stress, according to research [12]. It has been suggested recently that oxidative stress may play a role in the aetiology of lichen planus. Lipid peroxidation, which occurs when membrane-associated polyunsaturated fatty acids in phospholipids are oxidised, has long been thought to be a significant manifestation of oxidative stress [13]. The end product of lipid peroxidation, malondialdehyde (MDA), is a well-known marker of free radical-mediated damage and oxidative stress.

We found a considerably greater level of oxidative stress marker, serum MDA, in lichen planus patients compared to controls in this study. Our findings are consistent with those of Panchal et al [14], Shiva et al [15], Rekha et al [16] and Mansourian et al [17], who found significantly higher serum MDA levels in lichen planus patients ($p=0.001$) when compared to controls, confirming the previously reported link between oxidative stress and lichen planus. Similarly, Sander et al [18-19] that the LP patient group's MDA levels were greater than the control groups. Rai et al [20] also reported elevated MDA levels in LP, leukoplakia, and cancer. This is consistent with past studies that have found elevated MDA levels in LP patients. This shows that oxidative stress may enhance the formation of reactive oxygen species (ROS), resulting in increased lipid peroxidation. In addition, the dramatically altered status of antioxidant defence systems identified in our study underscored the involvement of oxidative stress in the pathogenesis of LP.

In the current investigation, individuals with LP had higher serum superoxide dismutase (SOD)

levels than healthy controls. This was in line with previous research by Aly DG [12], Abdel-Karim et al [21] and HassanI et al [22], who found significantly higher levels of serum SOD in patients with lichen planus when compared to controls, implying that oxidative stress, which produces ROS,

may play a role in the pathogenesis of LP. Uric acid has been found to be a protective antioxidant defence in patients with ischemic heart disease and other domains of monitoring, but its impact in patients with lichen planus has yet to be researched. Though antioxidants such as enzymes, vitamins, and others are important defensive mechanisms against oxidative stress, it is estimated that uric acid contributes to more than half of blood's antioxidant capacity. The patients' serum uric acid (UA) levels were considerably lower than the controls in our study. Sree et al [23] and Mohammed et al [24] had previously reported similar findings. Chakraborti et al [25] and Gupta et al [26], on the other hand, discovered considerably higher amounts of serum uric acid in lichen planus patients as compared to controls. Rekha VR et al [16] and Georgescu et al [27], on the other hand, found no significant variations in serum uric acid levels in lichen planus patients compared to control persons. Our findings revealed a statistically significant positive correlation between serum malondialdehyde (MDA) levels and superoxide dismutase (SOD), but a statistically significant negative correlation between malondialdehyde (MDA) levels and uric acid (UA). Abdel-Karim et al [21] discovered a substantial positive association between serum MDA and SOD, whereas Shiva et al [15] discovered a strong inverse correlation between serum uric acid and MDA.

Strengths and limitations of study

The strength of study relies with the fact that very few studies have been conducted for evaluation of oxidative stress in lichen planus in North Indian population. Therefore, numerous studies on large scale are required to probabilise the role of oxidative stress in pathogenesis of skin diseases especially Lichen Planus. Moreover, small sample size, limited time period, less patient followup and single centre are several restricting factor for this study. Hence, multi-centric studies on large scale might evaluate the patho-causative role of imbalance between the oxidant and antioxidant.

Conclusion

Recent evidence has clarified that ROS generation activates both physiologic and pathologic mechanisms that are not specific to the skin but should be considered as general responses to pro-oxidant stimuli, suggesting that oxidative stress and antioxidant imbalance may play a key role in the pathogenesis of skin diseases. Increased oxidative stress and an imbalance in the antioxidant defence mechanisms in lichenplanus may also play a role in the pathogenesis of lichen planus.

Conflict of Interest:	All authors declare no COI
Ethics:	There is no ethical violation as it is based on voluntary anonymous interviews
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