Oxidative Stress in Lichen Planus

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Abstract: Lichen planus (LP) is an inflammatory, papulosquamous disorder that affects the skin, mucous membranes, hair and nails. The exact pathogenesis is unknown. Oxidative stress and increased reactive oxygen species (ROS) and lipid peroxides have been implicated in the pathogenesis of LP. The aim: Is to evaluate the status of the oxidative stress and antioxidant defense system in patients with LP by measuring the serum nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD) levels and the erythrocyte catalase (CAT) levels. Patients and methods: Twenty LP patients and 20 healthy volunteers as controls that were ages- and sex-matched with the patients were included in the study. Serum levels of nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), and erythrocyte catalase (CAT) were measured. Results: We detected an increase in the serum levels of NO, SOD and the lipid peroxidation product MDA (P <0.000, P =0.005 and P =0.02, respectively) and a decrease in CAT levels in LP patients compared to controls (P =0.005) in our study. Oxidative stress was greater in mles than in females because MDA levels were increased (P =0.007) and erythrocyte CAT levels were decreased (P = 0.01). In addition, there was also a positive correlation between NO, MDA, and SOD and a negative correlation between erythrocyte CAT and the duration of LP. Conclusion: We concluded that increased oxidative stress and lipid peroxidation as well as an imbalance in the antioxidant defense system may play a role in the pathogenesis of LP.
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1. Introduction

Lichen planus (LP) is defined as a subacute, chronic dermatosis characterized by small, flat-topped, shiny and polygonal violaceous papules that may coalesce into plaques. It involves the skin, mucous membranes, genitalia, nails, and scalp. The clinical presentation of LP has several forms, including the actinic, hypertrophic, annular, erosive, follicular, linear, pigmented, and bullous types. It affects all races equally and presents mainly in the range from 30 to 70 years of age. [(1)] Recently it has been suggested that increased reactive oxygen species (ROS) and lipid peroxides may play a part in the pathogenesis of various skin diseases, such as atopic dermatitis, psoriasis, vitiligo and LP. [(2)]

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and the biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. [(3)]

The skin is particularly prone to insult of oxidative stress as it can be exposed to a degree greater than any other body tissue to excess heat or cold and UV radiation which not only has direct damaging effect but also trigger free radical chain reaction that may deplete skin antioxidant. [(4)]

Nitric oxide (NO) is a gaseous free radical that is released by the family of NO synthetase enzymes. It is a potent vasodilator, thus contributing considerably to the cardinal signs of inflammation. It is also known to exhibit cytotoxic effects in human skin. [(5)] There is compelling evidence that oxidative stress drives the production of oxidation products, such as malondialdehyde, which can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators, such as cytokines, which may be critical for the induction of some inflammatory skin diseases. [(6)]

The demonstration that the peroxisome proliferator- activated receptors, whose natural ligands are polyunsaturated fatty acids and their oxidation products may be involved in the pathogenesis of psoriasis or acne, has further strengthened the concept that ROS can drive the development of these disorders. [(7)] ROS trigger induction and maintenance of cutaneous inflammation. ROS may also participate in the pathogenesis of allergic reactions in the skin. [(8)]

Super oxide dismutase (SOD) is made by cell types such as fibroblast, lymphocytes. Removal of excess O_2 by SOD enzymes is important physiological antioxidant defense mechanism in aerobic organisms. [(1)]

2. Patients and Methods

This case control study was conducted on:-

1- Twenty patients suffering lichen planus (11females and 9 males). Their ages ranged from 20 to 75 years. They had different types of LP (classic, actinic, hypertrophic, and lichen planopilaris).

2- Twenty healthy volunteers, age and sex matched with the patient severed as controls.

All patients were subjected to:

Proper full history was taken, *general examination and local examination including:* site, shape and type of the diseases and external genital examination.

Blood samples were obtained from patients with LP and healthy controls after 12 hrs of fasting. The blood samples were centrifuged at 3,000 g for 5 min at 4 °C. Erythrocyte suspension was prepared by removing the buffy coat from the erythrocyte and diluting the remainder of the erythrocytes with 10 mL of 0.9% NaCl.

The resuspended erythrocyte, were then centrifuged at 3,000 g for 5 min and the upper layer was removed again. This was repeated 3 times and then diluted 4 times with water and mixed by vortex. Samples were stored at −40 °C until assayed.

Measurement of superoxide dismutase levels

SOD activity was determined in samples using reagents supplied by Randox Laboratories Ltd. (Crumlin, UK). This method employs xanthinexanthine oxidase (XOD) to generate superoxide radicals, which react with 2 iodophenyl-3-(4-nitrophenyl)-5-phenyltetrazolium (INT) chloride, to form a red formazan dye. The SOD activity in the sample hemolysate was then measured by the degree of inhibition of this reaction. The final color was measured at 505 nm and the results were expressed as SOD unit/g Hb.

Measurement of serum nitricoxide levels

Serum NO levels were assayed using the sandwich ELISA technique employing a kit from R&D systems (R&D Systems Inc. Minneapolis, MN). The assay determines NO concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. This reaction is based on the two-step diazotization reaction in which acidified NO2 produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azoderivative, which absorbs light as 540 nm.

Measurement of serum malondialdehyde levels

MDA was assayed spectrophotometrically by a kit supplied by OxisResearch (Portland, OR). The method is based on the reaction of the chromogenic reagent N-methyl-2-phenylindole (NMPI) with MDA at 45 °C. One molecule of MDA reacts with 2 molecules of NMPI to yield a stable carbocyanine dye, the absorbance of which is measured at 586 nm.

Measurement of serum catalase levels

Catalase is assayed spectrophotometrically by a kit supplied by OxisResearch. It is a two-step procedure. The rate of dismutation of hydrogen peroxide (H_2O_2) to water and molecular oxygen is proportional to the concentration of catalase (reaction 1). The sample containing catalase is incubated in the presence of a known concentration of H2O2. After incubation for exactly 1 min, the reaction is quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5,-dichloro-2-hydroxylbenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (HRP) in reaction 2. The resulting quinone-imine dye is measured at 520 nm (N-(4-antipyrl)-3-chloro-5-sulfonate pbenzo-quinonemonoimine).

Statistical analysis was carried out using SPSS 16.0. Results are presented as percentage, mean ±SD. Continuous variables were compared using *t* test, and categorical variables were analyzed with $\chi 2$ test. Correlation analyses were performed using Pearson. In all statistics, two-sided tests were used and the results were considered statistically significant at $P \leq 0.05$ and highly significant $P \leq 0.001$.

3. Results

This case control study was conducted on 20 patients suffering lichen planus (11females (%) and 9 (%) males). Their ages ranged from 20 to 75 years with mean 38.39 ± 11.77 years. They were age- and sex-matched with 20 healthy individuals that served as controls with a mean of 39.92 ± 11.79 years. The duration of the disease ranged from 3 months to 6 years with a mean ± SD of 1.46 ± 1.33 years (Table.1).

Regarding type of LP in the patient group, 13 (65.0%) patients had classical type, 2 (5.0%) patients had hypertrophic type, 2 (10.0%) patients had atrophic type, 3 (15.0%) patients had actinic type and 1 (5.0%) patients had actinic type (Tab.2).

The mean ± SD levels of serum NO (77.32 ± 12.133 lmol／L) and MDA (15.87 ± 3.022 lmol／L) in patients with LP were higher than those of the control group (P <0.0001and P =0.005, respectively). Serum SOD levels (16.1987 ± 3.012 U／mL) in patients with

LP were also higher than in healthy controls (P=0.02). In contrast, erythrocyte CAT levels (14171.47± 2788.29U) were significantly lower in the patient group than in the control group (P =0.005) (Tab.3).
Results showed a significant higher MDA and CAT in male patients compared to females (p=0.007, 0.01 respectively). No statistically significant deferens in NO and SOD in both sexes was detected (Tab.4).
A significant higher MDA and lower CAT were detected in patients with oral manifestations compared to patients without oral manifestations (p=0.02, 0.05 respectively). No statistically significant deferens in NO and SOD in both groups was detected (Tab.5).

Table (1): Age, Sex distribution and duration of illness of the study groups

	Study Group N 20		**Controle Group N=20**		***P***
	Range	**Mean±SD**	**Range**	**Mean±SD**	
Age	20-75	38.39 ± 11.77	21-76	39.92 ± 11.79	0.683
Duration of illness	3 m-6 ys	1.46 ± 1.33	-	-	-
	N	**%**	**N**	**%**	
Sex					1.00
Male	9	45	9	45	
Female	11	55	11	55	

Table (2): Types of LP in the patient group

	N	**%**
Classical	13	35.0
Hypertrophic	1	5.0
Atrophic	2	10.0
Actinic	3	15.0
Pigmented	1	5.0

Table (3): Comparison between study groups regarding Oxidative stress elements

	Study Group N = 20		**Control Group N =20**		**t-test**	***P* value**
	Mean	**±SD**	**Mean**	**±SD**		
NO (umpol/L)	**77.32**	**12.133**	**58.65**	**10.033**	5.3033	<0.0001 HS
MDA (U/ml)	**15.87**	**3.022**	**13.07**	**2.912**	2.9838	0.005 HS
SOD (U/ml)	**16.987**	**3.012**	**14.949**	**2.244**	2.4266	0.02 S
CAT (U)	**14171.47**	**2788.29**	**19155.49**	**6986.78**	2.9633	0.005 HS

Table (4): Comparison between male and females of the study group regarding Oxidative stress elements

	Males N = 9		Females N =11		t-test	*P* value
	Mean	**±SD**	**Mean**	**±SD**		
NO (umpol/L)	**72.42**	**11.033**	**79.15**	**10.933**	1.3640	0.1894 NS
MDA (U/ml)	**17.31**	**3.012**	**12.29**	**2.512**	4.0681	0.0007 HS
SOD (U/ml)	**16.997**	**3.012**	**16.841**	**2.934**	0.1169	0.9082 NS
CAT (U)	**14932.45**	**2766.29**	**11551.46**	**2986.78**	2.6021	0.01 S

Table (5): Comparison between patients with and without oral affection in the study group regarding Oxidative stress elements

	Oral affection N = 6		No oral affection N =14		t-test	*P* value
	Mean	**±SD**	**Mean**	**±SD**		
NO (umpol/L)	**79.60**	**12.159**	**70.14**	**10.938**	1.098	0.188 NS
MDA (U/ml)	**19.74**	**2.512**	**16.29**	**2.912**	2.519	0.02 S
SOD (U/ml)	**16.961**	**2.012**	**15.892**	**2.031**	1.0815	0.293 NS
CAT (U)	**11732.45**	**2766.29**	**14551.46**	**2786.78**	2.1773	0.05 S

A statistically significant positive correlation between NO ($r = 0.57$, $p = 0.005$), MDA ($r = 0.54$, $p = 0.005$), SOD ($r = 0.638$, $p = 0.001$), and the duration of illness, and a statistically negative correlation between CAT ($r = -0.48$, $p = 0.009$) and the duration of the disease was found. In addition, there was a significant positive correlation between NO and SOD and CAT and MDA (Tab.6).

Table (6): Correlation between studied elements (NO, MDA, SOD & CAT) and duration of illness in the Study group:

		NO	**MDA**	**SOD**	**CAT**
Duration of illness	**(r)**	0.573**	0.638**	0.328*	-.331*
	Sig. (2-tailed)	0.005	0.001	0.05	0.03
*. Correlation is significant at the 0.05 level **. Correlation is significant at the 0.01 level					

4. Discussion

Lichen planus (LP) is a unique, chronic, inflammatory skin disease that affects the skin, mucous membranes, nails and hair. [9] Increased reactive oxygen species (ROS) and lipid peroxides have been implicated in the pathogenesis of various skin diseases such as atopic dermatitis [10], psoriasis vulgaris [4], vitiligo [11]. Studies have reported an increased oxidative stress and lipid peroxidation in patients with lichen planus. [12; 13; 14] This suggested that reactive oxygen species may have a role in the pathogenesis of lichen planus. So the aim of the present study was to evaluate the status of the oxidative stress and antioxidant defence system in patients with LP by measuring the serum nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD) levels and the erythrocyte catalase (CAT) levels.

Results of the current study showed serum NO and MDA in patients with LP were higher than those of the control group (P <0.0001and P =0.005, respectively). Serum SOD levels in patients with LP were also higher than in healthy controls (P =0.02). In contrast, erythrocyte CAT levels were significantly lower in the patient group than in the control group (P =0.005).

Similar to our results, ***Sezer et al.,*** [12] found higher serum NO levels in patients with LP than in healthy subjects, suggesting that oxidative stress, resulting in generation of ROS, may play a role in the pathogenesis of LP. Also ***Aly and Shahin,***[13] the serum levels of NO were higher in patients with LP than in the control group.

Similar to our results, ***Sezer et al.,*** [12] found that serum MDA levels were significantly higher in patients with LP than in the control group. They suggest that oxidative stress may lead to increased production of ROS, thus resulting in increased lipid peroxidation. Also ***Sander et al.,*** [14] showed an increased staining intensity of the lipid peroxidation markers MDA and 4-hydroxynonenale in six patients with vulval LP compared with healthy controls. These findings indicated a reduced antioxidant defence and increased damage to lipids, DNA and proteins in LP, which is in accordance with our results. Moreover, ***Rai et al.,*** [15] reported high MDA levels in LP, leukoplakia, and cancer. Also ***Hassan et al.,*** [16] recorded higher serum MDA levels in patients as compared to controls. This suggests that oxidative stress may lead to an increased production of ROS, thus leading to increased lipid peroxidation and thereby increased levels of MDA.

SOD constitutes the first line of defence against oxygen-derived free radicals, converting the superoxide anion (O2–) into H_2O_2. CAT is the main enzyme involved in removing H2O2, which is generated from superoxide anion radicals by SOD. Similar to our results ***Hassan et al.,*** [16] found higher levels of SOD in LP cases compared to control. ***Sezer et al.,*** [12] found raised serum SOD levels and diminished serum CAT levels in patients with LP suggested that an imbalance in the antioxidant status may result in accumulation of H_2O_2, thus leading to vacuolization of the basal layer in LP.

Results of the current study showed significant higher MDA and CAT in male patients compared to females (p=0.007, 0.01 respectively). No statistically significant differences in NO and SOD in both sexes was detected.

Similar to our results, ***Aly and Shahin,*** [13], found a significantly high increase in the serum levels of MDA and a highly significant decrease in the serum levels of erythrocyte CAT levels in the male patients when compared to the females. However, NO and SOD showed an insignificant difference between the sexes. On the other hand, ***Ide et al.,*** [17] found that oxidative stress is greater in men than in women The mechanism by which females are thought to be more protected from the damaging effects of oxidative stress may be related to the antioxidant properties of estrogens. Moreover, estradiol has been documented as having antioxidant effects. [18]

Results of the current study showed significant higher MDA and lower CAT in patients with oral manifestations compared to patients without oral manifestations (p=0.02, 0.05 respectively). No statistically significant differentness in NO and SOD in both groups was detected.

Aly and Shahin, [13] also investigated the possible relation between the measured oxidative stress parameters and the clinical manifestations of LP. They found highly significant increase in the serum levels of NO, MDA, and SOD and a highly significant decrease in the serum level of erythrocyte CAT in patients that had oral involvement with their skin lesions were found.

To our knowledge, there were a few reported studies investigating the involvement of oxidative stress and antioxidant enzyme expression on oral LP patients. ***Anshumalee et al.,*** [19] reported that oxidative stress may play a role in oral LP. Meanwhile, in another study, the potent antioxidant lycopene was found effective in the management of oral LP. This therapeutic effect indirectly pointed to the role of oxidative stress in the pathogenesis of LP. [20]

Results of the current study showed a statistically significant positive correlation between NO (r = 0.57, p = 0.005), MDA (r = 0.54, p = 0.005), SOD (r = 0.638, p = 0.001), and the duration of illness, and a statistically negative correlation between CAT (r = −0.48, p = 0.009) and the duration of the disease was found. In addition, there was a significant positive correlation between NO and SOD and CAT and MDA.

Similar to our results, ***Aly and Shahin,*** [13] found a positive correlation between NO, MDA, SOD, and the duration of illness, and a negative correlation between CAT and the duration of the disease. However, in some studies, a negative correlation was reported between SOD activity and the duration of other diseases for which ROS is thought to be involved in the pathogenesis. [21; 22]

Conclusion

We concluded that increased oxidative stress and lipid peroxidation as well as an imbalance in the antioxidant defense system may play a role in the pathogenesis of LP.

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