Evaluation of the antioxidant status in patients of lichen planus in Kashmir valley – A hospital based study

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Lichen planus; Reactive oxygen species; Oxidative stress; Superoxide dismutase; Malondialdehyde; Reduced glutathione; Glutathione peroxidase; Nitric oxide

Abstract Background: Lichen planus is an inflammatory skin disorder of unknown etiology. Recently, increased reactive oxygen species (ROS) and lipid peroxides have been implicated in the pathogenesis of various inflammatory dermatoses including lichen planus.

Aims: The present study was designed to evaluate the oxidative stress and the antioxidant defense status in Kashmiri patients suffering from lichen planus.

Methods: A total of 60 patients with lichen planus (27 males, 33 females) and 60 control subjects, matched for age and gender were enrolled in this prospective case control study. Serum levels of Superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GPX) and Nitric oxide (NO) levels were measured in both patients and controls.

Results: Plasma levels of MDA, NO and SOD enzymes were significantly higher \((p<0.05)\) in patients than in controls. Plasma levels of GSH and GPX were significantly lower in patients than in controls \((p<0.05)\).

Conclusions: The findings in our study suggest an increased lipid peroxidation and an imbalance in the antioxidant defense mechanisms in lichen planus. This may play a role in the pathogenesis of lichen planus.

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1. Introduction

Lichen planus (LP) is an inflammatory, papulosquamous disorder that may affect the skin, mucous membranes, hair and nails. The initial description of LP was given by Hebra and later Erasmus Wilson (1869) gave it the name. The name ‘lichen planus’ has been derived from the Greek word ‘leichen’ (tree moss) and the Latin word ‘planus’ (flat) (Daoud and Pittelkow, 2003). Lichen planus is clinically characterized by faintly erythematous to violaceous, small, flat-topped, polygonal papules, distributed mainly on the flexor aspects of extremities, associated with intense pruritus. The oral cavity, genitals, nails and scalp may also be involved. Various clinical presentations of LP exist including the hypertrophic, atrophic, guttate, actinic, annular, linear, follicular, ulcerative (erosive), vesiculobullous and lichen planus pigmentosus. The exact pathogenesis is unknown, but cell-mediated immunity and humoral immunity have been implicated.
Activation of the cell-mediated immune response destined toward keratinocyte apoptosis is the prime event in the pathogenesis of LP. The process involves three sequential stages: LP-specific antigen recognition, cytotoxic lymphocyte activation, and keratinocyte apoptosis (Daoud and Pittelkow, 2003; Middel et al., 2000). Recently, increased reactive oxygen species (ROS) and lipid peroxidation have been implicated to have a role in the pathogenesis of various disorders like atopic dermatitis (Omata et al., 2001), psoriasis (Relhan et al., 2002), and vitiligo (Yildirim et al., 2003). Recent studies have reported an increased oxidative stress and lipid peroxidation in patients with lichen planus (Anshumalee et al., 2007; Sezer et al., 2007). This suggests that reactive oxygen species may have a role in the pathogenesis of lichen planus.

There is a paucity of data available in the literature regarding the antioxidant defense status in patients of lichen planus. So, the present study was designed to evaluate the status of oxidative stress and antioxidant defense system in patients of lichen planus belonging to ethnic Kashmiri population. This was done by measuring their serum levels of nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GPX).

2. Methods

This study was a hospital based case control study conducted in the Department of Dermatology, STD and Leprosy of SMHS Hospital (associated teaching hospital of Government Medical College Srinagar). A written informed consent was obtained from all patients and control subjects.

The present study included 60 ethnic Kashmiri patients with lichen planus attending our out-patient department. Patients who had received any systemic steroids or other immunosuppressive drugs were excluded from the study. Other exclusion criteria were patients with a history of trauma or any surgery one month prior to sampling, patients on NSAIDS, those suffering from any autoimmune disease or malignancy, and patients who were smokers.

The control subjects were 60 healthy individuals from the dermatology OPD, matched for age and gender.

Fasting venous blood samples (5 ml) were obtained from patients with LP and healthy controls and were drawn into vacutainers containing heparin as an anticoagulant. The blood samples were centrifuged at 3000 g for 5 min at a temperature of 4°C. Erythrocyte suspension was prepared by removing the buffy coat from the erythrocytes and diluting the remainder of the erythrocytes with 10 ml of 0.9% NaCl. The resuspended erythrocytes were then centrifuged at 3000 g for 5 min and the upper layer was removed again. This was repeated three times with water and mixed with Vortex. Samples of both the groups were then stored at a temperature of −40°C until analysis.

SOD activity was estimated by the method described by Kono (1978). Photo-oxidation of hydroxylamine hydrochloride was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazone, which was monitored at 560 nm. SOD enzyme of the sample removes the superoxide anion and inhibits the reduction. The level of this reduction was used as a measure of SOD activity.

GSH level was estimated by the method of Moron et al. (1979). In this method, 25% Trichloroacetic acid (TCA) was added to precipitate out and separate the protein by centrifugation at 2000 g for 15 min. GSH in the supernatant fraction forms a complex with 2,2-dinitro-5-S′-dithio-bis-nitrobenzoic acid (DTNB) which was measured at 412 nm.

MDA level in the plasma was estimated using the thiobarbituric acid (TBA) method, which measures the TBA reactive products chiefly the malondialdehyde (MDA) (Placer et al., 1996).

Plasma GPX was assayed according to the method of (Beutler, 1989).

In aqueous solution, NO rapidly degrades to nitrate and nitrite, therefore serum nitrate and nitrite levels were estimated as an index of NO production. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrate + nitrite) was measured using a spectrophotometer at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules (Cortas and Wakid, 1990). A standard curve was established with a set of serial dilutions of sodium nitrite. Linear regression was carried out using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations.

Statistical analysis of the data was performed by using Statistical Package for Social Sciences (SPSS Version 17) and inferences were drawn. Student’s t-test was used to determine the statistical significance of serum GSH, SOD, MDA, GPX and NO levels within both patient and control groups. A p-value of <0.05 was considered to be statistically significant.

3. Results

The present study included 60 patients of lichen planus, and an equal number of age and sex matched controls.

In the study group of 60 patients, there were 27 (45%) males and 33 (55%) females, with a female to male ratio of 1:0.82. The age of patients ranged from 8 to 60 yrs, with a mean ± SD age of 36.72 ± 11.97 yrs.

The control group comprised of 60 subjects (32 males and 28 females). The age of the controls ranged from 18 to 61 yrs with a mean age of 35.92 ± 11.84 yrs. The patients and controls were age and sex matched (p value < 0.05).

The clinical varieties of lichen planus noted in patients are delineated in (Table 1).

In the study group of 60 patients, seven patients had coexistent oral mucosal involvement. Among these seven patients with oral mucosal involvement, six had a reticular pattern and one had an erosive type of lichen planus.

Nail involvement was seen in just two of the patients, which was in the form of ridging and nail plate thinning.

Koebner’s phenomena were noticed in four of the patients.

Table 1: Table showing the clinical varieties of lichen planus.

<table>
<thead>
<tr>
<th>Clinical type of lichen planus</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical lichen planus</td>
<td>36 (60)</td>
</tr>
<tr>
<td>Hypertrophic lichen planus</td>
<td>11 (18.33)</td>
</tr>
<tr>
<td>Actinic lichen planus</td>
<td>4 (6.67)</td>
</tr>
<tr>
<td>Atrophic lichen planus</td>
<td>4 (6.67)</td>
</tr>
<tr>
<td>Guttate lichen planus</td>
<td>5 (8.33)</td>
</tr>
</tbody>
</table>
Our study revealed a higher plasma SOD activity in cases than in controls. The mean value of plasma SOD in cases was 5.32 ± 0.57 U/mL, while in controls, the mean value was 4.07 ± 0.99 U/mL. This difference was statistically significant with a p-value of <0.0001.

Plasma MDA levels were also found to be significantly higher in cases than in controls (mean value of 1.25 ± 0.37 nmol/ml in cases and 0.49 ± 0.23 nmol/ml in controls), with a p-value <0.0001.

In our study, we noticed a higher plasma NO level in cases than in controls. Mean value of plasma NO in cases was 6.41 ± 0.82 μmol/L, while in controls it was 5.58 ± 1.99 μmol/L, with a statistically significant difference (p-value <0.0001).

Plasma GSH levels were lower in cases as compared to controls. The mean value of plasma GSH in cases was 2.74 ± 0.6 nmol/ml as compared to 3.99 ± 2.81 nmol/ml in controls. The difference was statistically significant, with a p-value of 0.001.

Plasma GPX levels were lower in patients as compared to controls. Mean value of plasma GPX in patients was 47.32 U/L ± 2.46, while in controls, its mean value was 49.0 U/L ± 3.26, the difference being statistically significant (p-value <0.001).

The statistical analysis of the data is depicted in (Table 2).

4. Discussion

Skin is a major target for toxic insult by a broad spectrum of physical and chemical agents that are capable of altering its structure and function. Many environmental pollutants are either oxidants or catalyze the production of reactive oxygen species (ROS) directly or indirectly (Bickers and Athar, 2006).

Oxidative stress refers to a condition where an increased ROS production overwhelms the antioxidant defense mechanisms. It may be involved in the pathogenesis of many skin diseases such as melanoma and non-melanoma skin cancers (Sander et al., 2004a,b), psoriasis (Briganti and Picardo, 2003), vitiligo (Spencer et al., 2007), chronic urticaria (Okayama, 2005) and Behcet’s disease (Buldanlioglu et al., 2005). Recently, it has been proposed that oxidative stress may have a role to play in the pathogenesis of lichen planus (Sezer et al., 2007).

NO is a gaseous free radical and is known to exhibit proinflammatory and cytotoxic effects in the human skin (Ormerod et al., 1999). In the present study, we 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.

Lipid peroxidation, which results from the oxidation of membrane-associated polyunsaturated fatty acids of phospholipids, has been considered a major presentation of oxidative stress (Picardo et al., 1994). MDA, the end product of lipid peroxidation, is considered a good marker of free radical-mediated damage and oxidative stress (Kasperska-Zajac et al., 2008). In our study, we 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. Several other studies have also demonstrated increased MDA levels in patients of lichen planus (Sezer et al., 2007; Sander et al., 2004a,b).

SOD is an antioxidant enzyme that accelerates the dismutation of toxic superoxide radicals produced during the oxidative processes, into less harmful molecules, hydrogen peroxide and molecular oxygen (Koca et al., 2004). It is considered to be the first line of defense against oxidative stress. Our study revealed increased serum levels of SOD in patients as compared to controls. Our findings were in accordance with other studies (Sezer et al., 2007). This suggests that an imbalance in the antioxidant status may result in the accumulation of H2O2, thereby leading to vacuolization of the basal cell layer, as seen in LP.

GSH is regarded as a potent antioxidant and an enzyme cofactor and is under a tight homeostatic control maintained between GSH synthesis, its recycling from GSSG (oxidized Glutathione) and its utilization. Free radicals as well as other oxidative agents have been known to deplete GSH. In our study, we noticed lower plasma GSH levels in patients as compared to controls.

In our study, we also found a lower plasma GPX activity in LP patients as compared to controls.

Our study revealed that there is a definite impairment in the oxidative mechanisms in patients of LP, indicating the role of oxidative stress in its pathogenesis. Further studies need to be conducted in this regard, including a large number of patients suffering from different forms of disease, so as to confirm this opinion.

5. Conclusions

To conclude, oxidative insult has a role to play in the etiopathogenesis of LP. So, rational strengthening of the antioxidant defenses by the addition of antioxidants should be part of an optimal treatment strategy for such patients.

Conflict of interest

None.

Source of support

Nil.
References


