Evaluation of the antioxidant defense status in psoriasis

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Background: Psoriasis is a chronic inflammatory disease of the skin. The etiology of psoriasis is not known exactly. Recently, it has been suggested that an imbalance in the oxidant- antioxidant status due to an increased reactive oxygen species production and/or deficient function of the antioxidant system may be involved in the pathogenesis of psoriasis. The aim of this study was to evaluate the antioxidant defense status in patients with psoriasis and to look for a correlation, if any, between the antioxidant defense status and the severity of psoriasis.

Method: Sixty patients with psoriasis and an equal number of age and sex matched healthy controls were enrolled in the study. Plasma levels of malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD), and antioxidant potential (AOP) were measured in cases as well as controls.

Result: Patients with psoriasis were found to have significantly higher levels of plasma SOD ($P < 0.001$) and MDA ($P < 0.001$) and lower levels of GSH ($P < 0.001$) than controls. Plasma AOP was not significantly different between patients and controls ($P= 0.822$).

Conclusion: The results of our study support the hypothesis of an imbalance in the oxidant –antioxidant status in psoriasis, which could contribute to the pathogenesis of psoriasis.

Keywords: antioxidant potential, malondialdehyde, psoriasis, reduced glutathione, superoxide dismutase

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INTRODUCTION

Psoriasis is a chronic inflammatory and proliferative disease of the skin, the exact etiopathogenesis of which is still unknown. It is generally believed to have a multifactorial etiology in which several genes interact with one another and with the environmental stimuli to give rise to classical, sharply demarcated, erythematous, scaly plaques predominantly seen over extensor surfaces of the body [1]. One of the hypothesis for the pathogenesis of psoriasis is the imbalance between antioxidants and oxidants leading to oxidative stress. What lends credence to the antioxidant theory is the association in patients of psoriasis with various other conditions like cardiovascular disorders, diabetes mellitus and rheumatoid arthritis, all of which are associated with oxidative stress [2].

The skin is a major target of oxidative injury due to reactive oxygen species (ROS) like superoxide anions, hydroxyl, hydroperoxyl radicals, and hydrogen peroxide ions that originate in the environment and in the skin itself during various physiological and pathological processes. A complex of antioxidant enzymes in our body catalyze the reactions involving ROS scavenging. These enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In normal aerobic cells, there is a balance between oxidative damage and antioxidant protection. However, inadequate antioxidant protection or excess ROS production creates a condition known as oxidative stress which can contribute to the development

of disease. Reactive oxygen species arising in the environment and skin may damage cell compounds such as proteins, lipids, and DNA, resulting in increased lipid peroxidation and formation of malondialdehyde (MDA) [2].

It has been suggested that the increased ROS production and the deficient function of antioxidant systems activities may be involved in the pathogenesis of psoriasis [3]. Several studies have investigated the role of oxidants / antioxidants systems in psoriasis with discordant results [1-3]. The aim of this study was to evaluate the antioxidant defense system in psoriatic patients and look for a correlation between their levels and the severity of psoriasis.

PATIENTS AND METHODS

This hospital-based, case-controlled study was conducted in the Department of Dermatology, STD and Leprosy, SMHS Hospital, Kashmir, India (associated teaching hospital of Govt. Medical College, Srinagar J&K) from June 2009 to September 2010.

Inclusion criteria were as follows:

1. Patients with clinically typical psoriasis
2. Patients with psoriasis on topical therapy
3. Age ≥18 years
4. Kashmiri ethnicity

Exclusion criteria were as follows:

1. Receiving systemic therapy like retinoids, methotrexate, cyclosporine, or any other systemic medication
2. Associated systemic diseases like cardiovascular disease, diabetes, and hypertension
3. Malignancy
4. Smoking and alcoholism

The control group comprised patients who were visited in our department for some unrelated minor complaints or the patients' attendants.

Informed consent was obtained from both cases and controls and observations were recorded on a standard proforma.The study was approved by the ethical committee of the institute. A detailed medical history was taken which included age of onset, duration of disease, family history, any known aggravating factor, and a drug history. A thorough general physical, systemic, and cutaneous examination was carried out in all patients with emphasis on the clinical type, severity, and joint involvement. The severity of psoriasis was assessed according to the PASI (psoriasis area and severity index) score. A PASI score >10 were regarded as severe psoriasis and PASI ≤ 10 as mild psoriasis. Fasting venous blood samples were drawn from both groups (patients and controls) and plasma samples were extracted and stored at -20°C until analysis.

The GSH level was estimated by the method of Moron et al [4] in which TCA (Trichloroacetic), 2, 4-Dinitro-5-5′-Dithiobis-nitrobenzoic acid (DTNB) and centrifugation are used.The SOD activity was determined as described by Kono et al [5] in which hydroxylamine hydrochloride, nitro blue tetrazolium, and superoxide dismutase enzyme are used. The total amount of lipid peroxidation products in the plasma were estimated using the thiobarbituric acid (TBA) method which measures the TBA reactive products, chiefly malondialdehyde (MDA) [6]. The total antioxidant potential (AOP) was assessed by the modified method of Ozturk et al on the basis of determination of MDA levels before and after exposure to superoxide radicals produced by the hydroxylamine hydrochloride system [7]. The statistical analysis of the data was performed using SPSS computer program version 17. To evaluate the differences between the cases and controls, student's t-test was used. P- value less than 0.05 was considered statistically significant.

RESULTS

Sixty patients with psoriasis (32 males and 28 females) aged 19 to 70 years with a mean age of 37.07± 12.89 years were included in the study group. Sixty age and sex matched healthy individuals (32 males and 28 females) aged 18 to 61 years with a mean age of 35.91 ± 11.83 years were selected as controls. Of the 60 patients, 48 had plaque psoriasis, 9 guttate, 2 erythrodermic, and 1 had generalized pustular psoriasis. Two patients had concomitant psoriatic arthritis (Table 1). In patients, the median

Table 1. Morphological types of psoriasis in cases

Type of Psoriasis	Males	Females	Total
Chronic plaque	26	22	48
Guttate	5	4	9
Generalized Pustular	0	1	1
Erythrodermic	1	1	2
Total	32	28	60

Table 2. Plasma SOD* activity, MDA† level, GSH‡ level, and AOP§ in case and control groups

Parameters	Group	n	Mean	Std. Deviation	SEM‖	P-value
GSH (nmol/ml)	Cases	60	2.89	0.61	0.061	<0.001 S**
	Controls	60	3.99	0.42	0.054	
SOD (U/ml)	Cases	60	5.11	0.82	0.082	<0.001 S**
	Controls	60	4.07	0.32	0.042	
MDA (nmol/ml)	Cases	60	0.95	0.52	0.052	<0.001 S**
	Controls	60	0.49	0.04	0.005	
AOP (nmol-1/ml×h)	Cases	60	0.05	0.21	0.021	0.822 NS††
	Controls	60	0.06	0.11	0.015	

*-Superoxide dismutase, †- malondialdehyde, ‡- reduced glutathione, §- antioxidant potential, ‖ standard error of mean, ** significant, †† not significant.

duration of the disease was 2.75 years. The mean PASI score was 9.98 ± 7.97 (ranging from 0.80 to 38.20). The PASI score was more than 10 in 35 patients and less than or equal to 10 in 25 patients.

Plasma levels of MDA (0.95 nmol/ml vs. 0.49 nmol/ml, P < 0.001) and SOD enzyme activity (5.11 U/ml vs. 4.07 U/ml, P < 0.001) were significantly higher in psoriatic patients than in controls. Plasma levels of GSH (2.89 nmol/ml vs. 3.99 nmol/ml, P < 0.001) were significantly lower in patients than controls. There was no significant difference in the plasma levels of AOP (0.05 nmol/ml/hr vs. 0.06 nmol/ml/hr, P = 0.822) between patients and controls. There was no statistically significant correlation between the PASI score and the plasma SOD activity, MDA level, GSH level or AOP in the patient group. The results of the plasma levels of GSH, SOD, MDA and AOP are summarized in Table 2.

DISCUSSION

Psoriasis is a common chronic inflammatory skin disease characterized by a marked increase in the proliferation and abnormal differentiation of keratinocytes associated with prominent alterations in the dermal capillary vasculature and the presence of dermal and epidermal mononuclear leukocytes and neutrophils [8]. An increased capacity for chemotaxis and adhesion, and an increased ROS (reactive oxygen species) production in neutrophils have been reported in patients with psoriasis [3,8]. It has also been suggested that generation of ROS from neutrophils, keratinocytes, and fibroblasts can contribute to neutrophil activation, which plays an important role in the psoriatic process [9]. The increased ROS production during the inflammatory process in psoriasis, as a result of insufficient antioxidant mechanisms, may result in increased lipid peroxidation. In cell membranes, this process may lead to cell damage by continuing in chain reaction. It is also responsible for phospholipase A_2 activation, production of many mediators by arachidonic acid, and deactivation of adenyl cyclase and activation of guanylyl cyclase leading to a decrease in the cAMP/cGMP ratio, which is responsible for epidermal hyperproliferation [3].

Increased production of free radicals or ROS causes oxidative damage to biological molecules, cell membranes, and tissues. ROS induced oxidation of polyunsaturated fatty acids in biological systems results in the formation of lipid-peroxidation products such as malondialdehyde (MDA). Higher platelet, erythrocyte, tissue, serum and plasma levels of MDA, more plasma lipid peroxidation products, and a correlation with disease severity have been reported in patients with psoriasis previously [10-15]. In our study, we found statistically significant higher plasma MDA levels in patients with psoriasis than in controls (mean MDA in cases 0.952+0.052 versus 0.492+0.005 in controls, p-value < 0.001). Baz et al and Nassiri et al also reported increased levels of plasma MDA in patients with psoriasis [10,11]. Relhan et al found a significant difference in the plasma levels of MDA in psoriasis patients in the acute phase as compared to those in remission and normal controls. However, no significant difference was found in MDA levels between control subjects and patients in remission [12]. Yildirim et al reported increased levels of tissue, and not serum, MDA in patients with psoriasis [13]. In another study performed by Kokcam et al, plasma MDA levels were not different between psoriasis patients and controls, but MDA levels in RBC samples were significantly higher in psoriasis patients [14]. Nevertheless, all of these

reports have mentioned increased levels of MDA in patients with psoriasis, a finding similar to ours. However, no correlation was found between the levels of MDA and severity of psoriasis (PASI) in our study as observed in some of the previous investigations [10].

Reduced glutathione (GSH) is recognized as a potent antioxidant and enzyme cofactor and is under tight homeostatic control maintained between GSH synthesis, its recycling from GSSG (oxidized glutathione), and its utilization. Free radical and other oxidative agents are known to deplete GSH. In this study, we found lower plasma GSH (reduced glutathione) levels in psoriasis patients as compared to controls (the mean GSH was 2.898 ± 0.0619 in cases and 3.995 ± 0.0548 in controls, p-value < 0.001). This could possibly be due to an increased utilization of GSH for quenching ROS in psoriatic patients. Relhan V et al and Kokcam B et al and also reported decreased plasma GSH levels in psoriasis patients than in controls [12,14].

Superoxide dismutase, an antioxidant enzyme, accelerates the dismutation of toxic superoxide radicals produced during the oxidative energy process into less harmful molecules, hydrogen peroxide, and molecular oxygen. It has been suggested that an increased generation of superoxide anion radicals from neutrophils, and neutrophil accumulation in psoriatic lesions may cause abundant superoxide production during the phagocytic reaction and systemic activation of circulating neutrophils in psoriatic patients [15]. In our study, we detected a higher plasma SOD activity in psoriatic patients than in controls (mean plasma SOD activity in cases = 5.112+0.082, mean plasma SOD activity in controls = 4.073+0.042; p-value < 0.001). Baz K et al and Utas et al also reported higher plasma SOD activities in psoriasis patients which is in accordance with our study [10,16]. In fact, Therond et al found a higher SOD activity in fibroblasts and erythrocytes of psoriatic patients [17]. Some studies however have reported suppressed SOD activities in erythrocytes, neutrophils, tissues, and plasma [13,15].

Anti-oxidant potential (AOP) provides an overall indication of the total enzymatic and non-enzymatic antioxidant status. Severin et al and Nassiri et al reported that plasma total antioxidant capacity did not differ significantly between psoriasis patients and controls [11,18]. In our study, we also detected no significant difference in the plasma AOP between psoriatic patients and controls (the mean AOP was 0.0557± 0.0214 in case, and 0.0625± 0.0153 in controls; p-value was 0.822). However, Baz et al reported decreased plasma AOP in psoriatic patients as compared to controls [10]. As it has been unambiguously reported that there are increased levels of reactive oxygen species in psoriatics, it actually means that the antioxidant capacity, especially the inducible type, is definitely increased in psoriatics and consumed in quenching ROS. Hence, this observed picture of no apparent significant difference in total antioxidant potential in the plasma occurred.

The correlation between the severity of psoriasis and antioxidant status is controversial. Baz et al and Kural et al found no correlation between the PASI score and oxidative stress in patients with psoriasis [10,19]. Rocha-Pereira et al, through comparative study of the lipid profile and the oxidative status between controls and two groups of psoriasis patients (mild and severe), demonstrated that an imbalance between oxidants and antioxidants was also observed in mild psoriasis and worsening of psoriasis was associated with an increase in oxidative stress [20]. In our study, we noted no correlation between the PASI score and the plasma levels of GSH, MDA, SOD, or AOP in psoriasis patients. Although increased levels of ROS are definitely a comorbid factor in psoriasis, it is definitely not necessary that same should have a correlation with the PASI score.

We have to always keep in mind that the inducible antioxidant defense system is always active in quenching ROS which implies that if the severity of psoriasis and increased ROS do correlate, this inducible antioxidant defense system will also be active at same time to bring down the levels of ROS. Current evidence indicates that the interaction between genes and the environment are vital in the pathogenesis of psoriasis. Many environmental factors such as trauma, infection, oxidant drugs, alcohol and smoking have been linked to this disease, but the exact role of these factors in the etiology of psoriasis is still controversial. In our study, serum uric acid was not estimated and the two groups were not matched with respect to the nutritional status. The results of our study support the hypothesis of an imbalance in the oxidant-antioxidant status in psoriasis, which may have

a role in the pathogenesis of psoriasis; however, there is no relation between oxidant stress and the severity of psoriasis. Nonetheless, large case studies on other antioxidants such as uric acid are needed to establish the efficacy of antioxidants in the treatment of psoriasis.

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