Oxidative Stress in Patients with Scalp Seborrheic Dermatitis

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SUMMARY Seborrheic dermatitis (SD) is a common, chronic inflammatory skin disease that mainly affects the scalp. The objective of this study was to evaluate the activities of superoxide dismutase (SOD) and catalase (CAT) and the levels of malondialdehyde (MDA) in scraping samples of patients with scalp SD. Thirty consecutive patients with a diagnosis of scalp SD and thirty-one healthy volunteers were enrolled. The samples were obtained by scraping the skin surface of the scalp. SOD and CAT activities and MDA levels were measured in scraping samples by spectrophotometric method. SOD and CAT activities and MDA levels were significantly higher in patients than in controls ($p<0.001$ all). There was a positive correlation between the severity of the disease and itching scores (contingency coefficient = 0.671, $p<0.001$). Except for this correlation, there was no significant correlation among age, sex, duration and severity of the disease, itching scores, antioxidant enzymes and MDA levels in the patient group ($p>0.05$). Cutaneous oxidative stress in patients with SD may play an important role in the pathogenesis of the disease. Further clinical and laboratory evaluation of the oxidant/antioxidant system in patients with SD is warranted.

KEY WORDS: seborrheic dermatitis, oxidative stress, superoxide dismutase, catalase, malondialdehyde

INTRODUCTION

Seborrheic dermatitis (SD) is a common, chronic, recurring inflammatory skin disease that mainly affects the scalp and usually involves between one and three percent of the immunocompetent adult population. It is also seen on the face, chest and other body sites such as groins. Typically, the affected skin areas appear greasy and accompanied by red, scaling itchy rash. Seborrheic dermatitis is more common among adolescents and in males than in females. Therefore, these data suggest that hormones may play a role in its pathogenesis. Although a number of other factors besides hormones have been considered in the development of SD lesions, including *Malassezia* yeasts, sebum levels, immune response, neurologic diseases, psychological stress and dry air, the pathogenetic mechanism and risk factors for SD are still unclear

and so there is no effective treatment (1-4). Previously, the role of oxidative stress in the pathogenesis of various inflammatory skin diseases was shown (5,6). Therefore, oxidative stress might help in better understanding the pathogenesis and treatment of SD.

There are a few studies demonstrating an association of oxidative stress in plasma samples of SD patients (7-9). However, there are no literature data on the oxidative stress of the skin surface in the lesional area of patients with scalp SD. The aim of our study was to evaluate selected parameters of oxidative stress, superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), in scraping samples from patients with scalp SD.

MATERIALS AND METHODS

The local ethics committee of our university approved the study and each subject signed an informed consent form before entering the study. After obtaining the informed consent, scraping samples were obtained from the scalp of patients and normal controls. Data were collected over 8-month period, from June 2011 to January 2012. Clinical diagnosis of the disease was made by a dermatologist and confirmed by laboratory investigations and histology when required. Patients of either sex older than 18 years with mild to severe scalp SD (defined as having an Investigator's Global Assessment 1 to 4) involving 5%-25% of the scalp area were enrolled at a single hospital. A 4-grade scale (from 0 to 3) was used to evaluate the itching score. Patients with disease duration of more than twelve months, patients previously treated for SD, and those with other forms and localizations of the disease were not included. The patients and controls had no history of any topical or systemic drug therapy including antifungals, steroids, anticonvulsants, vitamins and anti-inflammatory drugs for at least three months before the study, and none of them had any other coexistent systemic or cutaneous diseases. None of the patients and controls showed signs of mental or physical symptoms of zinc deficiency. None of them had alcohol abuse problems and smoking habit. The females were not pregnant or lactating. Obese subjects and those testing positive for human immunodeficiency virus were not included in the study. In both groups, subjects had not washed their scalps and hair for at least three days before scraping sampling. The samples were obtained by a dermatologist by scraping the SD skin surface in patients and any scalp area in controls with

Table 1. Catalase (CAT) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels in scraping samples of patients with scalp seborrheic dermatitis and healthy controls

Parameter	Patients (n=30) Mean ± SD (median, minimum-maximum)	Controls (n=31) Mean ± SD (median, minimum-maximum)	p value
CAT (U/mg protein)	3.94±0.68 (3.96, 2.74-5.67)	1.86±0.58 (1.96, 0.73-2.83)	<0.001*
Female* (n=17/16)**	3.96±0.70 (3.82, 3.00-5.67)	1.82±0.50 (1.82, 1.02-2.75)	
Male* (n=13/15)**	3.92±0.68 (4.09, 2.74-5.02)	1.91±0.67 (2.03, 0.73-2.83)	
SOD (U/mg protein)	357.85±70.78 (354.24, 203.21-562.69)	172.54±42.71 (167.13, 121.76-330.26)	<0.001**
Female* (n=17/16)**	338.96±64.70 (336.90, 203.21-426.12)	165.78±36.81 (167.22, 127.92-260.48)	
Male* (n=13/15)**	382.56±73.22 (374.68, 291.14-562.69)	179.76±48.47 (167.32, 121.76-330.26)	
MDA (nmol/mg protein)	30.15±6.22 (29.65, 19.89-40.21)	16.81±4.52 (16.03, 10.07-29.33)	<0.001*
Female* (n=17/16)**	29.02±6.25 (27.44, 19.89-40.21)	17.24±4.12 (16.44, 12.69-25.77)	
Male* (n=13/15)**	31.63±6.10 (31.50, 21.59-39.78)	16.37±5.04 (16.03, 10.07-29.33)	

*Student's t-test; **Mann Whitney-U test; ***within each group, no statistically significant sex difference (Mann Whitney-U test, p>0.05 all)

a scalpel blade (without bleeding). Scraping samples were transferred into aliquots and stored at -70 ºC until use for determination of SOD and CAT activities and MDA levels.

Biochemical assays

Preparation of scraping homogenates

Scraping samples were homogenized with 1.15% ice-cold KCl in two volumes (wt/vol) of the same solution, using a Heidolph (Schwabach, Germany) 50110 R2R0 homogenizer. Biochemical assays were performed on the supernatant preparation in a Sorvall (Minneapolis, MN) RC-2B centrifugation of the homogenate at 14000 rpm for 30 min at +4 °C. Total protein content was determined by the method of Lowry *et al*. (10), using bovine serum albumin (E. Merck, Darmstadt, Germany) as a standard.

Measurement of CAT activity

CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler (11). Assay medium consisted of 1 M Tris HCl, 5 mM Na_2EDTA buffer solution (pH 8), 1 M phosphate buffer solution (pH 7), and 10 mM H_2O_2. CAT activity was expressed as U/mg protein.

Measurement of SOD activity

SOD activity was measured according to the method described by Fridovich (12). This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with p-iodonitrotetrazolium violet (INT) to form a red formazan dye, which was measured at 505 nm. Assay medium consisted of the 0.01 M phosphate buffer, 3-cyclohexilamino-1-propanesulfonic acid (CAPS) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with pH 10.2, solution of substrate (0.05 mM xanthine, 0.025 mM INT), and 80 U/L xanthine oxidase. SOD activity was expressed as U/mg protein.

Measurement of MDA levels

Lipid peroxidation level in scraping samples was expressed in MDA. Measurement was based on the method of Ohkawa *et al*. (13). The reaction mixture contained 0.1 mL of sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid, and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA). The mixture pH was adjusted to 3.5 and the volume was finally made up to 4.0 mL with distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1, vol/vol) were added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. MDA levels were expressed as nmol/mg protein.

Statistical analysis

Statistical analysis was carried out using the SPSS 17.0 for Windows statistical software. The conformability of quantitative data to normal distribution was examined using Kolmogorov-Smirnov test. As the age and sex were distributed normally, descriptive statistics was presented as mean ± standard deviation. Furthermore, descriptive statistics was also shown as median, minimum and maximum. The unpaired Student's t-test was used to compare the mean values between the groups. However, SOD activities were not in concordance with normal distribution, so Mann Whitney-U test was used in terms of groups. Contingency coefficient was used to compare the parameters such as duration, severity and itching score of the disease. The level of statistical significance was set at $p<0.05$.

RESULTS

A total of 30 patients with active SD (17 females and 13 males), mean age 34.5±10.8 (range 20-60, median 33) years were enrolled in the study. Control group included 15 males and 16 females, mean age 35.3±11.1 (range 19-58, median 35) years. The mean duration of SD was 5.5±2.6 (range 1-11) months. The mean severity of the disease was 1.7±0.6 (range 1-3, median 2). The mean itching score was 1.8.±0.6 (range 1-3, median 2). There were no significant between-group differences in the mean age and male to female ratio ($p>0.05$).

The activities of SOD and CAT and MDA levels in both groups and in females and males separately are shown in Table 1. All activities and levels were significantly higher in SD patients than in controls, includ-

Table 2. Relationship between disease severity and itching scores in patients with seborrheic dermatitis (n=30)

Disease severity	Itching score			
	(1)	(2)	(3)	Total
(1)	6	6	0	12
(2)	3	11	1	15
(3)	0	0	3	3
Total	9	17	4	30

Contingency coefficient=0.671, $p<0.001$.

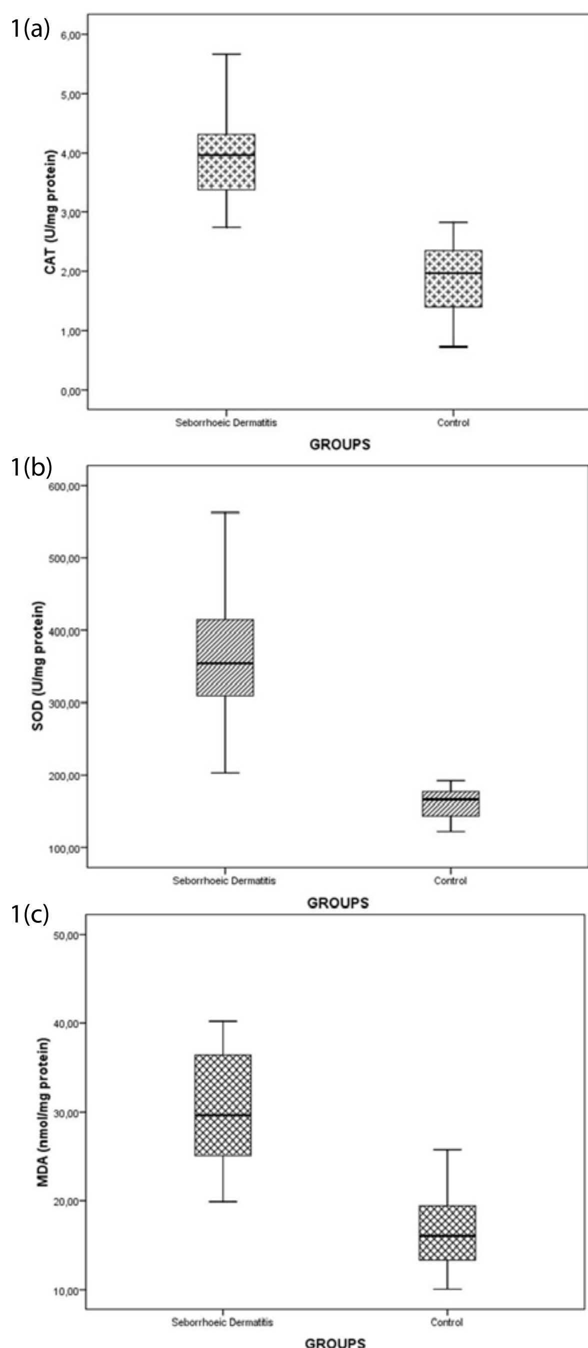

Figure 1. Box plot graphics: (a) catalase (CAT) and (b) superoxide dismutase (SOD) activities; and (c) malondialdehyde (MDA) levels in scraping samples of patients with scalp seborrheic dermatitis and healthy controls.

ing the results according to sex (p<0.001 all). Total results also are graphically presented in Figures 1 a-c. There was no statistically significant sex difference in either group (p>0.05 all). There was a positive correlation between the disease severity and itching score (contingency coefficient = 0.671, p<0.001) (Table 2). Except for this correlation, there was no correlation among age, sex, duration and severity of disease, itching score, antioxidant enzymes and MDA levels in the patient group (p>0.05).

DISCUSSION

The SOD-CAT system consists of the key antioxidant enzymes taking role in the defense against oxygen toxicity. SOD is an enzyme existing in the cytoplasm and providing the formation of hydrogen peroxide. It protects cells from the toxic effect of superoxide radicals. However, CAT destroys hydrogen peroxide and converts it to water and oxygen (14). At the same time, MDA is the final product and it is the best-known specific indicator of lipid peroxidation (15). Our results showed significantly elevated activities of SOD and CAT as well as MDA levels in scraping samples of lesions from patients with scalp SD compared to scraping samples of normal scalp of control subjects. In this study, the activity of CAT found to be twofold greater in patient group than in control group may be related to the higher production of superoxide radicals in SD lesion. Increased SOD activity may be due to the increased synthesis of the enzyme in response to the increased *in vivo* production of superoxide radicals. These results suggest that antioxidant defense of SD patients was higher compared to control group, possibly due to a compensatory response to oxidative stress, thereby protecting the cells against oxidative damage. When oxidative stress overwhelms the skin antioxidant capacity, the subsequent modification of cellular redox apparatus in patients with SD leads to an alteration of cell homeostasis and generation of degenerative processes (5,16). For this reason, reactive oxygen species may act as second messengers in the induction of antioxidant enzymes against oxidative stress as a biologic response. Also, increased MDA level in SD may show an indication of oxidative stress. It may play a role in the complex signaling network originating at the epidermal level due to a markedly altered chemical composition of all surface lipids in SD (17). We think that elevated oxidative stress may play a key role in understanding the pathogenesis of SD.

Previous two studies on oxidative/antioxidative system and SD were performed in the blood of patients (7,8). In these studies, SOD and MDA levels in patients with SD were not significantly different from those in healthy controls. In both studies, the severity or types of the disease were not specified and evaluated. On the other hand, in another recent study (9), the serum total antioxidant status in patients with SD was significantly higher than in control group. This last study included patients with different locations

of SD lesions and found no correlation between disease severity and serum total antioxidant status (9), whereas we investigated oxidative status only in our patients with scalp lesions. Because oxidative stress may be less detectable in the blood in the low severity skin disease, assessment of the oxidative stress parameters in the lesional skin area may be useful for better understanding of the SD pathogenesis. Nevertheless, we did not find any significant correlation between the severity of the disease and the oxidative stress parameters. This may also be due to the low severity of disease in our patients and the lack of patients with common lesions in this study. Therefore, new and detailed studies should be performed in peripheral blood and lesional area of patients with severe SD.

Furthermore, it is well known that topical zinc pyrithione and selenium sulfide are used for therapeutic management and prophylaxis of SD (2,18-20). Therefore, zinc and selenium can have important roles in human skin biology and epidermal differentiation. Zinc could be a physiological part of the oxidant defense system and an essential component of SOD (21,22). Lamore and Wondrak (23) have recently shown that zinc pyrithione impairs zinc ion homeostasis and upregulates stress response in keratinocytes. In addition, glutathione peroxidase and thioredoxin reductase are two natural antioxidant enzymes that contain selenium and depend upon selenium activity for their antioxidant functionality (24,25). The successful clinical studies with topical zinc pyrithione or selenium sulfide applications in SD may be due to their antioxidative effects.

In our study, both male and female SD patients showed highly significant differences in the oxidative stress parameters compared to control male and female subjects. However, when we compared the males and females within the same group (both control and patient groups), there was no significant sex difference. This finding suggests that the local oxidative stress in patients with SD may be independent of sex and sex hormones. Unfortunately, the effects of hormones and sex were beyond the scope of the present study. Therefore, in the future, this issue should be investigated in more detail in properly designed studies.

We evaluated the severity of disease and itching score in the lesions. As expected, we found a correlation between itching scales and severity of the disease. However, there was no correlation of itching scores with antioxidant enzymes and MDA levels in the patient group. The precise role of oxidative stress in the pathogenesis of pruritus is still unclear. Itching may be increased due to the severity of inflammation in patients with SD.

CONCLUSION

Due to the limited number of patients included in our study, we believe that new studies with more patients are needed. The disease duration in our patients was less than twelve months and none of them was immunocompromised. Only the scalp form of SD was included in our study. Owing to these limitations in the present study, no definitive conclusion can be drawn. Further clinical and laboratory evaluation of the oxidant/antioxidant system in patients with SD is warranted. Our results showed that elevated local oxidative stress may play an important role in the pathogenesis of SD regardless of patient sex. It appears likely that these changes in scraping samples of SD lesions are not the cause, but might be the consequence of cutaneous inflammation. Oxidative stress may originate from the abnormal epidermal physiology and inflammation of the skin. We thought that this situation in the lesional area may show adaptation of the body to the oxidative stress. However, the exact role of oxidative stress in SD may be clarified only in large and detailed studies.

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