Oxidants and Anti-Oxidants Status in Acne Vulgaris Patients with Varying Severity

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Abstract. Acne vulgaris is a common dermatological disorder with a multifactorial pathogenesis. Oxidative status has been implicated in the pathogenesis of several skin diseases, including acne. This study was aimed to investigate the levels of oxidative stress biomarkers in acne vulgaris patients with varying severities. The study involved 156 patients with acne and 46 healthy human controls. Based on clinical examination, patients were grouped into 3 subgroups as follows: mild, moderate, and severe acne. Oxidative stress was examined by measuring plasma levels of catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA). Plasma levels of MDA in acne patients were significantly higher as compared with that of the controls, whereas activities of the antioxidant enzymes SOD and CAT were lower. Moreover, TAC was also low in acne patients as compared with that of the controls. Higher MDA levels in the severe acne subgroup as compared with that of the mild and moderate subgroups were also observed. Furthermore, in the severe acne subgroup, a significant negative correlation was observed between MDA and CAT levels. The data suggests that oxidative stress plays a key role in acne progress and may be employed as a biomarker index to assess the disease's activity and to monitor its treatment.

Key words: Acne vulgaris, catalase, superoxide dismutase, total antioxidant capacity, malondialdehyde

Introduction

Acne vulgaris (acne) is a common dermatological condition typically occurring in adolescence and late childhood. It is associated with multiple causative factors and a complex pathogenesis. Follicular hyperkeratinization, sebaceous hyperplasia, and bacterial colonization are the main factors underlying the occurrence of acne, and inflammation and immune reactions play a crucial role in its pathogenesis as well [1].

The pathogenesis of acne involves *Propionibacterium acnes*, which appears to play an initiating role by producing low-molecular-weight chemotactic factors that lead to the accumulation of neutrophils at the site of acne comedones [2]. Following phagocytosis, these neutrophils release inflammatory factors, such as lysosomal enzymes, with resultant damage to the follicular epithelium [3]. Furthermore, they also generate several potent reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and others [4], which cause tissue injury at the sites of inflammation [4,5]. These oxidants are well known for inducing oxidative damage in lipid molecules, resulting in a chemical insult to the surrounding healthy tissues. Neutrophil-generated ROSs are also known to be closely correlated with the pathogenesis of a variety of inflammatory skin diseases [6,7].

Antioxidant defense systems, such as the enzymes superoxide dismutase (SOD) and catalase (CAT), keep ROS production in check, thereby maintaining an appropriate cellular redox balance. Alterations in this redox balance resulting from elevated ROS levels and/or decreased antioxidant levels can lead to oxidative stress [8]. Lipid peroxidation, a process of oxidative degeneration of polyunsaturated fatty acids that is set into motion by ROS, leads to the formation of highly reactive aldehydes, such as malondialdehyde (MDA), and may be one of the reasons for cellular membrane damage [8].

In this study, I measured the levels of oxidative biomarkers in the plasma of patients with acne vulgaris

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Table 1. Levels of oxidative biomarkers in acne patients and controls

	Acne (n = 156)	Controls (n = 47)	F	P
CAT U/mL	0.4±0.2	0.6±0.1	46.72	< 0.0001**
SOD U/mL	28.1±9.5	59.9±3.8	506.45	< 0.0001**
TAC μmol/mL	1.2±0.4	1.9±0.2	172.47	< 0.0001**
MDA nmol/mL	1.4±0.3	0.6±0.1	369.45	< 0.0001**

(**): Highly statistically significant difference

Table 2. Levels of oxidative biomarkers in patients with varying severities of acne

	Acne severity		
	Mild (n = 46)	Moderate (n = 83)	Severe (n = 27)
CAT U/mL	0.4±0.2	0.4±0.2	0.5±0.3
SOD U/mL	28.8±8.7	29.5±10.5	22.6±4.5[#,$]
TAC μmol/mL	1.3±0.3	1.3±0.4	1.1±0.3[#,$]
MDA nmol/mL	1.3±0.4	1.4±0.2	1.6±0.3[#,$]

([#,$]): Statistically significant when compared to mild or moderate acne subgroups, respectively

in Saudi Arabian patients and compared them with respective levels in healthy controls. Further, I assessed the correlations between the levels of plasma oxidative biomarkers and disease severity.

Materials and Methods

Subjects. I assessed 156 patients with acne vulgaris (82 males and 74 females; mean age, 20.2±3.6 years). All patients were recruited from Outpatient Dermatology Clinics affiliated to Qassim University, KSA. Signed and informed consent was obtained from all the subjects prior to the initiation of the study. The study was approved by ethical review committee, College of Medicine, Qassim University.

Patients with varying disease severities (mild, moderate, and severe) were included in the study. The Global Acne Grading System was used to assess the clinical severity of acne [9]. Briefly, the total severity score was derived from the summation of 6 regional sub-scores, which were derived by multiplying the factor for each facial region (1 for the chin and nose, 2 for the forehead and each cheek, and 3 for the chest and upper back) by the most heavily weighted lesion within each region (1 for ≥1 comedone,

2 for ≥1 papule, 3 for ≥1 pustule, and 4 for ≥1 nodule). The regional factors were derived based on the surface area, distribution, and density of pilosebaceous units. Based on this system, patients were graded into 3 groups as follows: mild, moderate, and severe acne [9]. Of the 156 patients evaluated, 46 patients were graded as mild, 83 were graded as as moderate, and 27 patients were graded as severe. In addition, 47 healthy, unrelated subjects (29 males and 18 females; mean age, 20.1±3.3 years) from the same local region were selected as controls. Neither the patients nor the controls had received any topical and/or systemic drug therapy for at least 3 months prior to blood collection; none of them had any other coexistent disease.

Five mL of peripheral blood was collected in EDTA tubes after 10–12 h of fasting. Plasma was separated by centrifugation at 2000 rpm for 5 min and stored at -80°C in aliquots for further use.

Determination of CAT and SOD activities in plasma. The activity of CAT in plasma was determined by adding 0.1 mL of plasma to an equal amount of 30% H_2O_2 and incubated for 3 min. Then, 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone were added and the produced color was measured at 520 nm [10].

Table 3. *Correlation analysis of oxidative markers in the plasma of acne patients*

	CAT	SOD	TAC	MDA
CAT	1			
SOD	0.005223	1		
TAC	0.07076	0.216947	1	
MDA	-0.07148	-0.14293	-0.05597	1

The plasma activity of superoxide dismutase (SOD) was measured according to the method described by Nishikimi et al. [11]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of the nitroblue tetrazolium dye, and the produced color is measured at 560 nm.

Determination of plasma total antioxidant capacity. The level of plasma total antioxidant capacity (TAC) was measured by incubating 0.02 mL of plasma with 0.5 mL of reaction substrate for 10 min at 37°C. Then, the chromogen solution was added and incubated for further 5 min. The change in color was measured at 505 nm [12].

Determination of plasma level of MDA. The lipid peroxidation end product, malondialdehyde (MDA), was measured in plasma according to the procedure described by Ohkawa et al. [13]. The reaction mixture contained 0.1 mL of plasma, 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid, and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. A mixture of n-butanol and pyridine (15:1, v/v) were added and the solution was mixed vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm.

Statistical analysis. Results of the oxidative markers were analyzed by using one-way ANOVA, followed by *Tukey's* test to calculate the significance. Data are reported as mean value ± standard deviation. The comparison was considered to be statistically significant or highly significant when p value is less than 0.05 or 0.001, respectively.

Results

The results for the levels of oxidative stress biomarkers measured in the plasma of the acne patients are shown in **Table 1**. The mean activities of CAT and SOD as well as the levels of TAC were significantly lower in the plasma of acne patients as compared to that of those in the control group (p<0.0001). In

contrast, the mean level of lipid peroxidation measured as MDA was significantly higher in the acne patients as compared to that of those in the control group (p<0.0001).

Table 2 illustrates the plasma levels of oxidative markers for the mild, moderate, and severe acne subgroups. Results indicated that there was no significant differences in CAT levels in patients with acne of varying severity (p>0.05). However, the SOD levels were significantly lower in the severe acne subgroup as compared to that of those in the mild and moderate subgroups (p<0.05). Similarly, the levels of SOD and TAC were significantly lower in the plasma of severe acne patients as compared to the levels in the mild and moderate acne patients (p<0.05). In contrast, the levels of the lipid peroxidative product MDA were significantly higher in the severe acne subgroup as compared to the levels of those in the mild and moderate subgroups (p<0.05).

The correlation between the levels of plasma oxidative markers in patients with acne of differing severities is shown in **Table 3**. A positive correlation was observed between the activities of SOD and CAT in the plasma of acne patients. Further, a positive correlation between the levels of TAC and CAT activity in the plasma of acne patients was noted. Interestingly, the levels of lipid peroxidation were found to be negatively correlated with the activities of CAT or SOD as well as with the TAC level in the plasma of acne patients. Therefore, further correlation studies were performed to investigate the effects of acne severity on the levels of oxidative and antioxidative biomarkers. As shown in **Figure 1A-I**, a significant correlation between CAT activity and MDA levels in the plasma of patients with severe acne was observed. However, no significant correlation was found in the mild and moderate acne subgroups.

Discussion

Acne vulgaris frequently occurs in the second decade of life. The pathogenesis of this disease is multifactorial. Compelling evidence suggests that oxidative stress is involved in the onset of acne [14]. In acne breakouts, changes occur in the content of
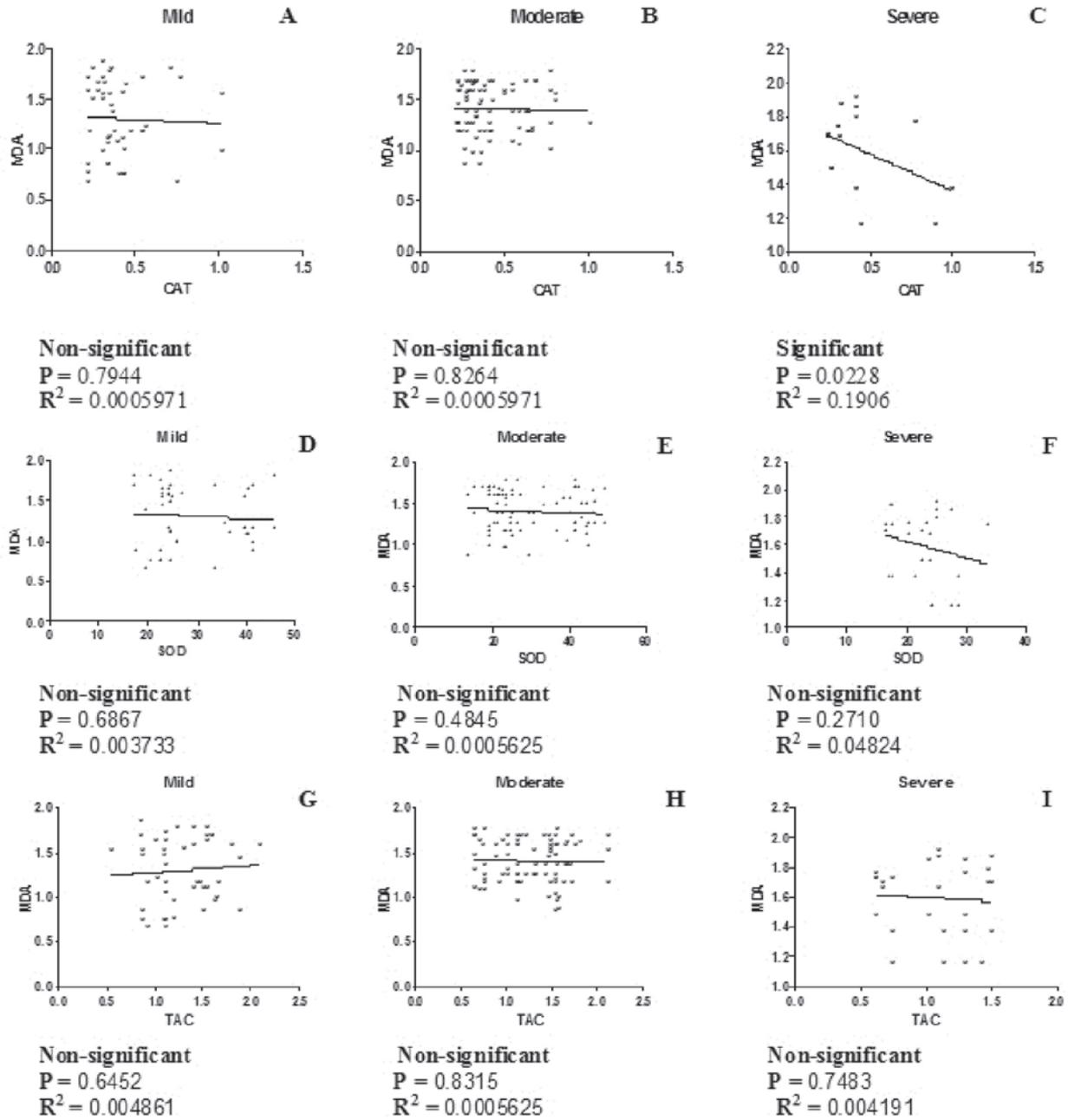
Figure 1. *Correlations between oxidative stress biomarkers in the plasma of patients with mild, moderate, and severe acne.*

sebum as well as in the rate of sebum release from the sebaceous glands; further, the release of ROS from affected follicular walls may lead to the progressive inflammatory reactions in acne [15].

Human cells have both enzymatic and nonenzymatic antioxidant defense systems. The SOD-CAT system is a major enzymatic system that acts as the first line of defense against oxygen-derived free radicals; it controls ROS production by catalyzing the dismutation of the superoxide into hydrogen peroxide, which is further converted into water by catalase and is thus crucial in maintaining an appropriate cellular redox balance [16]. Alterations in this normal balance, which may occur due to elevated ROS production and/or decreased antioxidant levels, can lead to a state of oxidative stress [8,17].

The results of the current study demonstrated a significant elevation of plasma lipid peroxide levels in acne patients as compared to levels in healthy controls. In contrast, the activities of plasma SOD and CAT as well as the level of total antioxidant capacity (TAC) were found to be significantly diminished in acne patients as compared to those in healthy individuals. Previous studies have revealed that the level of lipid peroxidation increases in inflammatory diseases; moreover, its final product, MDA, is considered an indicator of the oxidative stress in the cells [18]. Thus, the high plasma levels of MDA in our acne patients may be a result of cellular damage caused by ROS. These results suggest that acne is mediated, at least in part, by the increased generation of ROS, which may be attributed to reduced levels of antioxidant enzymes, including SOD or CAT. In various diseases, it has been observed that the SOD-CAT system may be affected in a way of increasing and decreasing or in two different directions [16].

Similar to present findings, Sarici et al. reported that serum levels of MDA in patients with acne vulgaris were significantly higher than those of the controls. Also, they revealed significantly lower SOD and CAT activity in the patient group than in the control group [19]. Moreover, Akamatsu et al. reported that lipid peroxide levels were significantly increased in patients with acne [20]. However, in contrast, Basak et al. found no statistical differences in MDA levels between acne patients and normal individuals [7]. These two different results may be explained by the fact that the antioxidant system undergoes various interactions with environmental or genetic factors that affect its increase, decrease, or normal levels.

Also, results revealed the association between the higher levels of oxidative stress biomarkers and the severity of acne among Saudi Arabian patients. The MDA levels were significantly elevated in the plasma of patients with severe acne patients as compared to those in patients with mild and moderate acne. Moreover, SOD activity and TAC levels were significantly decreased in the plasma of patients with severe acne as compared to the levels of patients with mild or moderate acne. No statistically significant difference was observed in CAT activity levels among patients with differing disease severity.

In contrast to present results, Basak et al. did not identify a correlation between acne severity and the levels of antioxidant enzymes or MDA in the leukocytes [7]. We assumed that this disagreement was most likely attributed to the difference in the source of samples used for measuring oxidative biomarker levels. In plasma, the levels of these markers do not indicate the origin of their production [21].

Data also revealed a significant negative correlation between CAT and MDA levels in patients with severe acne **(Figure 1C)**, while no such significant correlation was noted for patients with mild or moderate acne. Moreover, no significant correlation was observed between SOD activity, TAC activity, and MDA levels in acne patients, irrespective of disease severity. These findings clearly showed that oxidative stress is involved in acne and may play an important role in its pathogenesis and development [20]. Toxic molecules and reactive oxygen species play a crucial part in the pathogenesis and severity of acne vulgaris. The causative microorganism for the acne causes release of the chemotactic factors, which result in the accumulation of neutrophils. This process in turn causes damage to the follicular epithelial cells because of the release of the inflammatory factors like lysosomal enzymes. *Reactive oxygen species* are also released from the neutrophils in the inflamed tissues. The augmentation of ROS resulted in exhaustion of the antioxidant enzymes, leading to the reduction of their plasma level.

The alterations in oxidant/antioxidant status noted in this study may be utilized as a biomarker index for differentiating between varying levels of severity of acne and for evaluating the response to treatment. This suggestion needs to be confirmed by conducting further studies on a larger number of acne patients. The alterations in the antioxidant enzyme activities in the plasma of acne patients might reflect a peripheral response of the organism to increased oxidative stress. However, when antioxidants levels are measured in plasma, it is not possible to determine the origin of these enzymes [21]. It appears likely that these changes are not the cause but rather the consequence of cutaneous inflammations such as acne [22]. Thus, antioxidant oral supplementation or topical application may be an effective approach in improving the efficacy or avoiding the potentially damaging effects of the

therapeutical agents. Further research is required for optimal planning of acne therapy, and it may be useful to include at least one antioxidant drug along with the currently used acne treatment combinations.

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