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SHORT COMMUNICATION

# Oxidative Stress in Patients With Acne Vulgaris

Ozer Arican,[1] Ergul Belge Kurutas,[2] and Sezai Sasmaz[1]

[1]*Department of Dermatology, Kahramanmaras Sutcuimam Medical Faculty, Kahramanmaras, TR-46000, Turkey*
[2]*Department of Biochemistry, Kahramanmaras Sutcuimam Medical Faculty, Kahramanmaras, TR-46000, Turkey*

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Acne vulgaris is one of the common dermatological diseases and its pathogenesis is multifactorial. In this study, we aim to determine the effects of oxidative stress in acne vulgaris. Forty-three consecutive acne patients and 46 controls were enrolled. The parameters of oxidative stress such as catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD), and malondialdehyde (MDA) in the venous blood of cases were measured spectrophotometrically. The values compared with control group, the relation between the severity and distribution of acne, and the correlation of each enzyme level were researched. CAT and G6PD levels in patients were found to be statistically decreased, and SOD and MDA levels were found to be statistically increased ($P < .001$). However, any statistical difference and correlation could not be found between the severity and distribution of lesions and the mean levels of enzymes. In addition, we found that each enzyme is correlated with one another. Our findings show that oxidative stress exists in the acne patients. It will be useful to apply at least one antioxidant featured drug along with the combined acne treatment.

## INTRODUCTION

Acne vulgaris is one of the common dermatological diseases frequently found in late childhood and adolescence [1]. Sebaceous hyperplasia, follicular hyperkeratinization, and bacterial hypercolonization, as well as immune reactions and inflammations may lead to acne, which has a quite complex pathogenesis [2]. Propionibacterium acnes produces follicular lipases, proteases, and hyaluronidases, several enzymes that may play an important role in the inflammatory process [3]. Besides, many researchers think that acne pathogenesis can not exactly be understood.

In acne, sebum produced by sebaceous glands, content changes and reactive oxygen species (ROS) may be released from the impacted damaged follicular walls; at the same time it is thought that this may be the reason for the progress of the inflammation in the pathogenesis of the disease [4]. It is also known that some of the drugs used commonly in the treatment of acne function by decreasing ROS [5].

Oxygen, which is an important and vital component for human, can produce reactive types (superoxide anion, hydrogen peroxide, and hydroxyl radicals) known as ROS. These radicals are formed with the reduction of oxygen to the water. Normally, the production of these radicals is slow and they are removed by the antioxidant enzymes existing in the cell. Superoxide dismutase (SOD), catalase (CAT), and glucose-6-phosphate dehydrogenase (G6PD) are some of the important antioxidant enzymes. Malondialdehyde (MDA) is the end product of lipid peroxidation and one of the indicators of oxidative stress. When SOD and CAT enzymes are insufficient for oxidative stress, ROS denotes its impact by starting the lipid peroxidation on the membranes of organs and cells.

The human epidermis represents the first barrier against infective agents and the serum is easy to obtain from patients and small amount of it will suffice. The changed antioxidant enzyme activities of erythrocyte in the patients compared to healthy controls might be a peripheral response of the organism to increased oxidative stress. It can be put forward that increased antioxidant enzyme activities may reflect a preceding cellular oxidative stress or serve as compensatory mechanism. Although acne vulgaris is the most frequent disease of the young population, only a few studies on antioxidative system in acne pathophysiology have been performed up to now [6, 7]. In this study, levels of some antioxidant enzymes in erythrocyte in a group of patients with acne vulgaris were measured and compared with control group, and then the relation between the severity and distribution of acne lesions and the levels of these enzymes in blood was researched. Since the serum levels are easily affected by many different factors [8], these enzymes have been studied in erythrocytes different from those used in previous studies [6, 7].

Correspondence and reprint requests to Ozer Arican, Department of Dermatology, Kahramanmaras Sutcuimam Medical Faculty, Kahramanmaras, Turkey; ozerari@gmail.com, drozer@ksu.edu.tr

## MATERIALS AND METHODS

Forty-three acne patients including 30 women, and 46 healthy controls including 28 women were included in this study. Consecutive acne patients, who came to our dermatology clinic, were enrolled. Both patients and controls had no history of any topical and systemic drug therapy included vitamins and anti-inflammatory drugs at least 3 months prior to blood collection, and none of them had any other coexistent disease. None of them had alcohol abuse problems and smoking. Prior to initiation of the study, each subject was informed about the aim of the study and signed an informed consent. Only the cases with comedonal lesions were recorded as mild severity, the cases with papule and pustule as moderate severity, and the ones with nodulo-cystic lesions as severe [9]. The distribution of lesions in patients includes face and body (fore and/or back) covering.

Patients and controls were between 13 and 35 (mean: 20±4.3) and between 14 and 31 (mean: 21±4.2) years old, respectively. Twenty-eight patients demonstrated only face covering and the others demonstrated both face and body covering. Seven out of total cases were mild, 31 cases showed moderate severity, and 5 cases were severe.

All blood samples, taken after 10–12 hours of fasting in the morning between 08:30 and 10:30 hours. The blood from forearm vein was collected into 5 mL vacutainer tubes containing potassium ethylen diamine tetra acetate (EDTA). The blood samples were centrifuged at 1000 xg for 10 minutes at 4°C to remove plasma. The buffy coat on the erythrocyte sediment was separated carefully after plasma was removed. The erythrocytes were washed three times with 0.9% NaCl solution to remove the plasma remnant. After each procedure, erythrocyte-saline mixture was centrifuged at 1000 xg for 10 minutes at 4°C. The haemolysates were prepared from the washed cells to measure the parameters of biochemical workup.

CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler [10]. Assay medium consisted of 1 M Tris HCl, 5 mM $Na_2$EDTA buffer solution (pH 8), 1 M phosphate buffer solution (pH 7), and 10 mM $H_2O_2$. CAT activity was expressed U/g hemoglobin.

SOD activity was measured according to the method described by Fridovich [11]. This method employs xanthine and xanthine oxidase to generate superoxide radicals which 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-propanesulfonicacid (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/g hemoglobin.

G6PD activity was determined at 37°C according to Beutler [10]. The reaction mixture contained 1M Tris-HCI pH 8, 6 mM glucose-6-phosphate sodium, 2 mM nicotinamide adenine dinucleotide phosphate (NADP), 0.1 M $MgCl_2$, and haemolysate in total volume of 3 mL. One unit of enzyme activity is the amount catalyzed by the reduction of 1 mM of NADP per minute. Results were expressed as U/g hemoglobin.

Lipid peroxidation level in the plasma samples was expressed in MDA. It was measured according to procedure of Ohkawa et al [12]. The reaction mixture contained 0.1 mL sample, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid, and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. The mixture pH was adjusted to 3.5 and the volume was finally made up to 4 mL with distilled water and 5 mL of the mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the absorbance of the organic layer was measured at 532 nm. Results were expressed as nmol/mL.

The hemoglobin level was measured with Spectronic-UV120 spectrophotometer by the method of cyanomethemoglobin. Bovine serum albumin was used as a standard.

Statistical assessments were carried out with SPSS 10.0 packet program. All data were given as mean ± standard deviation (SD). Chi-square test was used to compare differences between the frequencies. The unpaired $t$ test was used to compare mean values between groups. Spearman correlation test was used for the assessment of correlation. Statistical difference was taken as $p < 0.05$.

## RESULTS

Age and sex distributions of patients and control groups were determined to be similar ($p > 0.05$). Mean values of the levels of SOD and MDA were significantly higher in patients as compared to controls (4108±661 (2800–5700) versus 2654±520 (1755–3800) U/g Hb, $p < 0.001$ and 3.9±0.60 (2.9–5.2) versus 2.1±0.29 (1.5–2.7, 2.1) nmol/mL, $p < 0.001$, resp.). On the contrary, mean values of the levels of CAT and G6PD were significantly lower in patients as compared to controls (8.5±1.22 (6.4–11.1) versus 13.8±2.31 (9.9–18.6) U/g Hb, $p < 0.001$ and 6.7±1.01 (5.2–9.1) versus 10.0±2.02 (6.4–16.4) U/g Hb, $p < 0.001$, resp.). The results also are shown in Figure 1. However, any statistical difference and correlation could not be found between the severity and distribution of the disease and the levels of each of them ($p > 0.05$). There was no correlation among ages or sexes and serum antioxidant enzymes and MDA levels in both groups ($p > 0.05$). Correlation among disease severity, and serum levels of antioxidant enzymes and MDA are presented in Table 1.

## DISCUSSION

ROS are toxic molecules and play critical roles in many of the inflammatory skin diseases [4, 13]. Propionibacterium acnes taking part in acne pathogenesis cause the release of some chemotactic factors leading to neutrophils

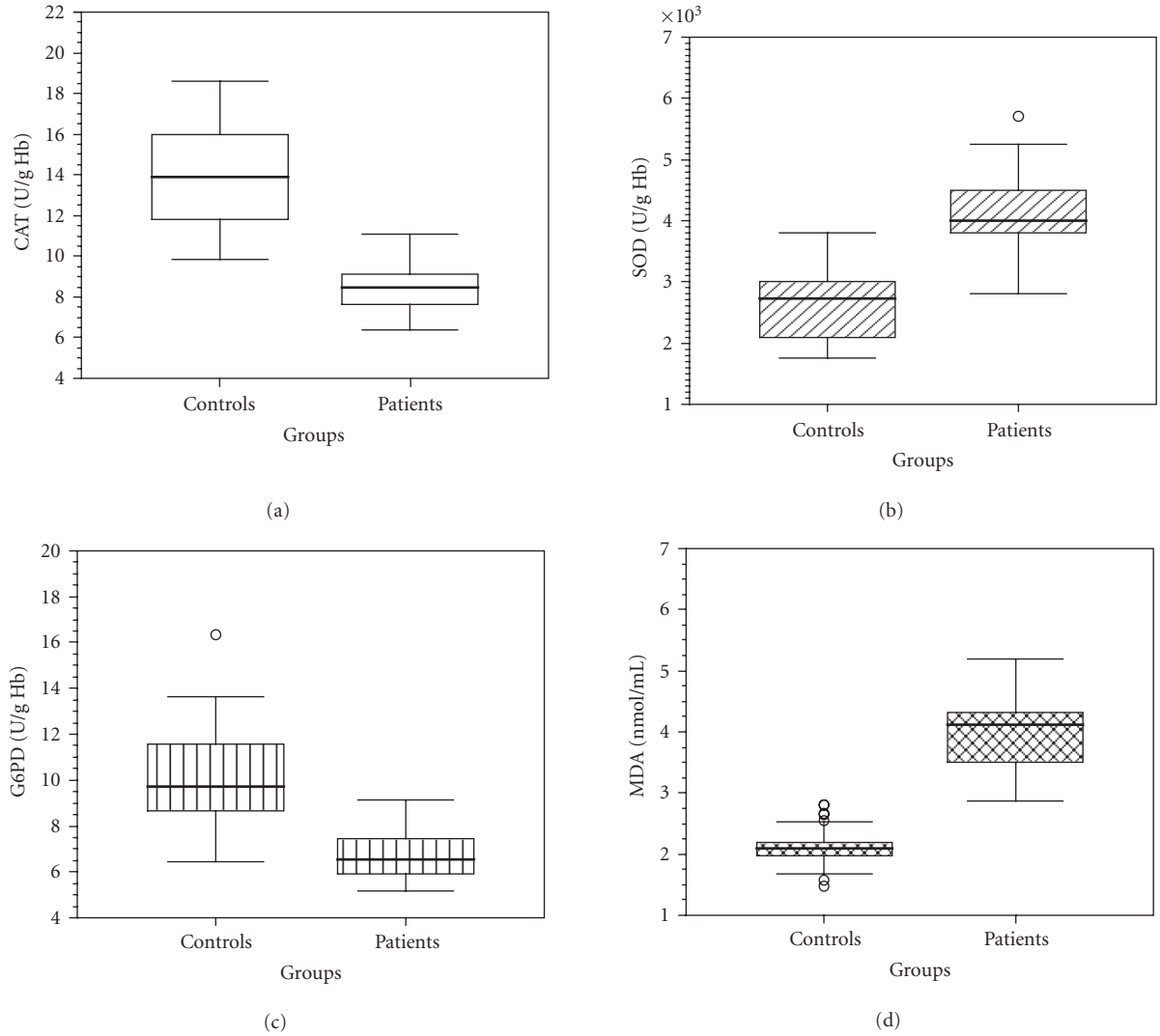


Figure 1. (a) CAT, (b) SOD, (c) G6PD, and (d) MDA serum levels of acne patients and controls in box plot graphics (○: Outliers).

Table 1. Correlation among disease severities and serum levels of antioxidant enzymes and MDA evaluated by the Spearman rank correlation.

| Severity and enzymes | Statistical values | SOD | CAT | G6PD | MDA |
|---|---|---|---|---|---|
| Severity | $R$ | 0.097 | −0.105 | −0.049 | 0.20 |
| | $P$ | 0.53 | 0.50 | 0.75 | 0.18 |
| SOD | $R$ | — | −0.617 | −0.569 | 0.733 |
| | $P$ | — | < 0.001 | < 0.001 | < 0.001 |
| CAT | $R$ | — | — | 0.623 | −0.807 |
| | $P$ | — | — | < 0.001 | < 0.001 |
| G6PD | $R$ | — | — | — | −0.606 |
| | $P$ | — | — | — | < 0.001 |

accumulation, and this situation causes damages to follicular epithelia after the release of some inflammatory factors such as lysosome enzymes as a result of phagocytosis. ROS is released from the active neutrophils in the inflammatory tissue. These oxidants attack DNA and/or membrane lipids and cause chemical damage to them, including the healthy tissue [4, 14, 15]. Squalene, which is specific to human sebum, protects skin surface from lipid peroxidation, while its lipid peroxidation products lead to comedogenic effects, and they have been specified in open or closed comedones as highly concentrated [4].

Antioxidant effects of drugs such as tetracycline, erythromycin, mynocycline, and metronidazole, which are used in acne treatment, attract attention and these effects give superiority to them and make them more preferable compared to the other antibiotics [5, 6, 14]. Metronidazole, one of them, does not have any antibacterial effect [16]. On the other hand, benzoyl peroxide, a topical agent for the treatment of acne, shows the ability to induce an inflammatory reaction mediated by oxidative stress in addition to its antibacterial activity [4].

SOD-CAT system consists of antioxidant enzymes taking role in the defense against oxygen toxicity [17]. SOD is an enzyme existing in cytoplasm and providing the formation of hydrogen peroxide. However, CAT destroys hydrogen peroxide [18, 19]. In various diseases, it has been observed that SOD-CAT system may be affected in a way of increasing and decreasing or in two different directions [17]. In our study, erythrocyte SOD activity increased in patients with acne; on the other hand erythrocyte CAT activity decreased. It is thought that superoxide anion radicals, which are produced abundantly in the cell, inhibit CAT. In addition, it has been demonstrated that superoxide anion nitrites also inhibited CAT [20]. Therefore, the decrease in the level of CAT enzyme makes us think that hydrogen peroxide accumulates in the cell. As a parallel finding to this, Akamatsu et al found that hydrogen peroxide level increased in patients with acne [14]. It may be thought that SOD level increased as a reaction to oxidant stress occurring as a result of oxidant/antioxidant imbalance in the cell. Basak et al found SOD activity low but CAT activity high in leukocyte [6]. We found that leukocytes SOD activity in acne patients was lower than in controls in our other study [7]. Thus, we think that SOD-CAT system may be affected differently in erythrocytes and leukocytes.

The indicator of the oxidative stress in the cell is the level of lipid peroxidation and its final product is MDA. There are some studies providing that the level of lipid peroxidation increases in inflammatory diseases [21]. This may evidence damage to ROS. Thus, high levels of MDA in our patients may evidence the damage caused by ROS. in our patient group. Basak et al found it normal in their acne group [6]. These two different results lead us to think that the antioxidant system may be affected in a way of increasing, decreasing, or remaining in the normal limits against one or several effects and it could be explained in further studies.

In our study it was seen that G6PD in our patient group is statistically low compared to in the control group. G6PD is the key enzyme of the pentose phosphate pathway. NADPH produced by the enzyme plays a role in protecting oxidant/antioxidant balance in the cell and reducing the oxidative stress [22]. Besides, NADPHs are necessary nucleotides in the formation of reduced glutathione in erythrosine, reduction of methemoglobin to oxyhemoglobin and CAT activities [23]. The decrease in CAT activity may be related to the increase in superoxide radicals or the decrease in G6PD activities. Therefore, in the recent studies, it has been shown that CAT enzyme has a tetrameric structure and its each monomer ties one molecule NADPH [24].

On contrary to our expectations, we did not find an increasing level of activity in these enzymes and MDA as a result of the disease's being common and severe. Basak et al could not find a connection between the severity of the disease and the level of enzymes, either [6]. This proves that oxidant/antioxidant balance may be affected to a specific extent in the organism. But the enzyme levels could not be influenced by the total amount of factors or agents that expose the organism. This idea may be confirmed by further investigations. In addition, we found each enzyme is correlated to one another. These results may be useful in the treatment and long-term follow-up of the disease.

These findings clearly indicate that oxidative stress exists in the acne and may play an important role in its pathogenesis. The changed antioxidant enzyme activities of erythrocyte in the patients might be a peripheral response of the organism to increased oxidative stress. However, when antioxidants levels are measured in serum, it is not possible to determine the origin of these enzymes. The question remains whether antioxidant enzymes abnormalities represent the proverbial egg or chicken dilemma in acne. It appears likely that these changes are not the cause, but might be the consequence of the cutaneous inflammation such as acne. When we consider that successful clinical results have been achieved with topical NADH practiced on rosacea, which attracts attention with its similar clinical findings [25], it may be thought that patients suffering from acne may benefit from these kinds of antioxidant treatments. 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. In planning acne therapy, synergistic effects should be considered by selecting anti-acne agents which have different mechanisms of action and targets on the pathogenesis of acne. Until new products are developed, it will be useful to prefer at least one antioxidant featured drug along with the combined acne treatment.

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