Evaluation of biomarkers of oxidant–antioxidant balance in patients with acne vulgaris
Menha A. Ibrahim, Manal E. Helmy, Hanan H. Sabry, Suzan M. Farouk, Lubna Y. Ebrahim, and Eman R. Amer

*Department of Dermatology and Venereology, Benha University, Egypt
†Department of Dermatology and Venereology and ‡Department of Clinical Pathology, Benha Teaching Hospital, Benha, Egypt

Correspondence to Menha A. Ibrahim, MD, Department of Dermatology and Andrology, Benha Faculty of Medicine, Benha University, Benha, Egypt
Tel: + 20 106 078 6979; fax: + 20 224 026 818; e-mail: kuran101@hotmail.com

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Background
There have been controversial arguments and conflicting research results on whether oxidative stress plays a role in the etiopathogenesis of acne vulgaris, or it is just a consequence of the disease.

Objectives
The aim of this study was to explore the effect of oxidative stress burden, mainly lipid peroxidation, on the oxidant–antioxidant balance and on the development of acne by studying the activity of some oxidative stress markers.

Patients and methods
This study was conducted on 40 acne vulgaris patients and 36 age-matched and sex-matched healthy controls. Patients were classified into mild, moderate, severe, and very severe on the basis of the Global Acne Grading System. Venous blood samples taken from both patients and controls were analyzed to determine the activity of the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glucose-6-phosphate dehydrogenase (G6PD); the levels of malondialdehyde (MDA), a major by-product and an important marker of lipid peroxidation, were also determined. Different laboratory techniques suitable for each marker were used and the results were read spectrophotometrically.

Results
A highly significant decrease in the mean blood G6PD, CAT, and SOD enzyme activities (P<0.001) and a highly significant increase in the mean MDA (P<0.001) blood levels were detected in acne patients compared with controls. None of the measured markers (G6PD, SOD, CAT, and MDA) was found to correlate with patients’ age, disease duration, or severity (P>0.05).

Conclusion
The significant decrease in the antioxidant enzyme biomarkers, as well as the significant increase in the lipid peroxidation marker, indicates the compromised oxidant–antioxidant balance in patients with acne vulgaris. This justifies adding antioxidants to acne therapy and calls for finding a multibiomarker scoring system for a better evaluation and monitoring of oxidative stress and its consequences.

Keywords:
acne vulgaris, biomarkers, oxidative stress

Introduction
Acne vulgaris is one of the most common dermatologic diseases. Hormonally mediated sebum overproduction, follicular hyperkeratinization, Propionibacterium acnes hypercolonization, chronic inflammation of the pilosebaceous unit with consequent immune reactions, genetic susceptibilities, and various environmental factors have been linked to the pathogenesis of this multifactorial condition [1].

It is widely accepted that P. acnes plays a role in the development of inflammatory acne lesions, but whether it starts inflammation by itself or other mechanisms are involved is yet to be clear. On exposing epidermal cells to P. acnes in vitro, reactive oxygen species (ROS) production (oxidative burst), particularly superoxide anions (O_2^-), takes place accompanied by a series of events including the release of proinflammatory molecules, IL-8, and apoptosis. Thus, production of superoxide anions by epidermal keratinocytes can initiate P. acnes-induced inflammation of the skin [2].

Free radical-mediated reactions have been implicated in the etiopathogenesis of acne, but their role in eliciting this response and their contribution to disease progression remain unexplored [3]. Oxidant–antioxidant imbalance usually associates chronic inflammation, as that taking place in acne vulgaris [4]. Studies show how oxidative stress blood markers serve to highlight the impact that oxidative stress imposes on acne disease [3,5]. These studies provide indirect yet very obvious indicators of mismatch between
an oxidative stress burden and a diminished antioxidant defense system capacity in acne patients.

Free radicals such as superoxide anion (O$_2^{-}$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (•OH) have very short half-life, which makes it difficult to determine their levels; indirect evaluation of their levels is the alternative solution. This is accomplished by measuring antioxidant enzymes [such as glucose-6-phosphate dehydrogenase (G6PD), catalase (CAT), and superoxide dismutase (SOD)] that deactivate such toxic free radicals or convert them into less toxic ones. Another way is to measure the levels of products of oxidative stress, such as malondialdehyde (MDA), which is a major by-product of lipid peroxidation and one of the most frequently used biomarkers to investigate the oxidative damage of lipids [6].

G6PD enzyme has a unique role as a principal source of NADPH, which is the main intracellular reducing agent that promotes the antioxidant action by combating the damaging effect of free radicals produced by peroxidases [7].

The SOD-CAT system consists of antioxidant enzymes taking a role in the defense against ROS. SOD dismutes the superoxide anion to form O$_2$ and H$_2$O$_2$. CAT, a tetrameric structure with each monomer binding one molecule NADPH, finishes the job by deactivating H$_2$O$_2$, converting it to oxygen and water. This takes place regularly, a step that protects the skin from the damage induced by free radicals [8,9]. The aim of this study was to investigate the effect of oxidative stress, mainly lipid peroxidation, on the oxidant–antioxidant balance and on the development of acne, by studying the activity of some markers of oxidative stress – namely, SOD, CAT, G6PD, and MDA levels.

**Patients and methods**

This study was conducted on 40 acne vulgaris patients and 36 age-matched and sex-matched healthy controls, both recruited from the outpatient clinics of Benha University and Benha Teaching Hospitals over a period of 6 months from October 2012 to April 2013. The study was approved by the Research Ethics Committee of Benha Faculty of Medicine. All participants were nonsmokers and had not received any medications or supplements, including antioxidants, during the last 3 months before the start of the study. Those who complained of any other disease were excluded from the study.

Informed consent was obtained from each of the participants, who were subjected to the following examinations:

1. Thorough history taking including acne history, duration, and history of previous therapy.
2. Thorough clinical examination to exclude any coexisting disease.
3. Dermatological examination to assess acne type, location, and severity. Lesions were scored according to the GAGS [10], whereby each lesion is given a value depending on severity: no lesion = 0, comedones = 1, papules = 2, pustules = 3, and nodules = 4. The score for each area (local score) is calculated using the formula: local score = Factor x Grade (0–4). The global score is the sum of local scores. A score of 1–18 is considered mild; 19–30, moderate; 31–38, severe; and greater than 39, very severe.

**Laboratory analysis**

Venous blood samples were taken for laboratory tests. Each blood sample from the patients and from the control group was distributed into two heparinized tubes. In one tube hemoglobin and G6PD measurements were carried out immediately. The other tube was centrifuged at 3000g for 10 min. Plasma was collected carefully and stored at −20°C until the time of analysis of plasma MDA and CAT. Red blood cells were washed with normal saline, and its hemolysate was prepared for erythrocyte SOD estimation.

Hemoglobin was measured using a cell counter (Sysmex KX-21 N; Sysmex America, Inc., Mundelein, Illinois, USA). Whole-blood G6PD was determined spectrophotometrically according to the method of Beutler et al. [11] in which G6PD catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate to 6-phosphogluconate and reducing NADP to NADPH. The increase in the absorbance of NADPH, for reduction of NADP, is proportional to the activity of the G6PD in the sample, which was detected at 340 nm.

Erythrocyte SOD was determined spectrophotometrically using the commercial Biorex method according to Arthur and Boyne [12]. This method uses xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.

The SOD activity is then measured by the degree of inhibition of this reaction at 505 nm. Plasma CAT was measured according to the spectrophotometric method by Göth et al. [13]. The yellow complex formation between H$_2$O$_2$ and ammonium molybdate was measured at 405 nm.

Plasma MDA levels were measured according to the method of Gérard-Monnier et al. [14], which is based on the reaction of a chromogenic reagent N-methyl-2 phenylnindol with MDA at 45°C, to form a colored carbocyanine dye with a maximum absorption at 586 nm.

**Statistical analysis**

Data were collected, tabulated, and statistically analyzed using SPSS, version 16 software (SPSS Inc., Chicago, Illinois, USA). Data were presented as mean ± SD. Student’s *t*-test was used for comparison of two parametric means. Spearman’s correlation coefficient (*r*) was used for assessment of association between two variables. A *P* value of 0.05 or less was considered significant.
Results
The patients' ages ranged from 15 to 25 years (mean = 19.4 ± 3.1 years). There were 22 female and 18 male patients. The mean duration of illness in the patient group was 3.1 ± 2.3 years. The mean clinical severity score was 36.7 ± 16.9.

There was a significant decrease (P<0.001) in the mean activity of the three antioxidant enzymes erythrocyte G6PD, plasma CAT, and erythrocyte SOD in the patient group compared with that in controls. The mean plasma level of MDA in the patient group was significantly increased (P<0.001) compared with that of the control group (Table 1).

None of the activity of each of the three antioxidant enzymes, G6PD, CAT, SOD, as well as MDA, showed significant correlation with either age, duration of acne vulgaris, or severity score (P>0.05) (Table 2).

There was no significant correlation between plasma MDA levels and activity of each of the three antioxidant enzymes G6PD, CAT, and SOD (P>0.05) (Table 3).

Discussion
In this study, the biomarkers of ROS-induced damage in acne vulgaris patients were measured in the acne patients and controls; the results showed a significant decrease in blood G6PD, CAT, and SOD activity levels in acne patients compared with their levels in the control group. In contrast, MDA levels were statistically significantly increased in patients compared with controls.

These results are in agreement with those of Bowe et al. [1], who found decreased blood SOD and glutathione peroxidase (GSH-PX) activity in papulopustular acne patients. They attributed the lowered levels of these antioxidant enzymes to the depletion at a faster rate by the chronic inflammation that characterizes acne. Abdel Fattah et al. [3] reported significant decrease in SOD activity both in blood and tissue in severe acne patients as compared with mild and moderate ones and controls. They also reported a significant increase in blood MDA levels in acne patients compared with controls. The present study confirms the previous work of Mofalah et al. [15], who detected a significant increase in tissue MDA levels in acne patients, which, in contrast to the present results, correlated positively to disease severity. In addition, a study by Sarici et al. [16] on parameters of oxidative stress in acne patients revealed a significant decrease in serum SOD and CAT activity and increased MDA.

In two consecutive studies, Arican et al. [5] measured acne patients’ levels of oxidative stress markers, both in erythrocytes [4] and leukocytes [17]. G6PD and CAT levels were decreased and MDA levels were increased in both studies. In contrast, SOD levels showed significant increase in erythrocytes and a significant decrease in leukocytes. They thought that the SOD-CAT antioxidant system might behave differently in erythrocytes and leukocytes. Likewise, lowered leukocyte SOD activity was reported by Basak et al. [18]. The decrease in G6PD activity in the current acne patients group might compromise the antioxidant status in the epidermis knowing that G6PD is the rate-limiting enzyme in the pentose-phosphate pathway, which produces NADPH. Although other sources for NADPH exist, studies have shown that G6PD is the major source of NADPH, which is required by many enzymes, including enzymes of the antioxidant pathway; hence, it is important in maintaining the oxidant–antioxidant balance in the cell, and subsequently in reducing the oxidative stress status [19]. NADPHs produced by G6PD are also needed for the synthesis of reduced glutathione (GSH) and thioredoxin systems to regenerate reduced forms that will then have roles as antioxidants [20]. A study of G6PD-deficient mice revealed that induced inhibition of G6PD in mice led to increased ROS and apoptosis, whereas inducing G6PD overexpression completely prevented the increase

### Table 1. Laboratory parameters of acne vulgaris patients and controls

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Patients (n=40)</th>
<th>Controls (n=36)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (U/g Hb)</td>
<td>9.61 ± 3.1</td>
<td>14.6 ± 1.98</td>
<td>8.486</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>1361.1 ± 129.8</td>
<td>1773.6 ± 307.1</td>
<td>7.758</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Catalase (MU/l)</td>
<td>75.7 ± 35.2</td>
<td>110.8 ± 13.6</td>
<td>5.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MDA (μmol/l)</td>
<td>0.59 ± 0.32</td>
<td>0.27 ± 0.09</td>
<td>6.01</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

G6PD, glucose-6-phosphate dehydrogenase; MAD, malondialdehyde; SOD, superoxide dismutase.

*P<0.05 is considered statistically significant.

### Table 2. Correlation between laboratory parameters and clinical data in acne vulgaris patients and controls

<table>
<thead>
<tr>
<th>G6PD (μg/g Hb)</th>
<th>SOD (μg/g Hb)</th>
<th>CAT (μg/g Hb)</th>
<th>MDA (μg/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>−0.20</td>
<td>−0.20</td>
<td>−0.156</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>−0.20</td>
<td>−0.12</td>
<td>−0.19</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Severity score</td>
<td>0.16</td>
<td>0.24</td>
<td>−0.105</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

G6PD, glucose-6-phosphate dehydrogenase; MAD, malondialdehyde; SOD, superoxide dismutase.

### Table 3. Correlation coefficient between malondialdehyde and other laboratory parameters

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (μg/g Hb)</td>
<td>0.003</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>SOD (μg/g Hb)</td>
<td>−0.29</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Catalase (U/g Hb)</td>
<td>−0.008</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

G6PD, glucose-6-phosphate dehydrogenase; SOD, superoxide dismutase.
in ROS levels, which suggests that G6PD is critical for the regulation of redox homeostasis in the body [21]. This also suggests that G6PD can serve as a marker that reflects the level of oxidative stress; moreover, the oxidative stress status can be ameliorated by increasing G6PD expression and activity.

The significant decrease in CAT enzyme levels in the acne patients’ plasma in this study can be explained by the overproduction of H₂O₂ by neutrophils in acne lesions [22], as part of the oxidative stress process. Naini et al. [8] concluded that CAT enzyme gets deactivated by the high level of H₂O₂ released as part of the imbalance between the oxidative stress and the antioxidant enzyme systems. Therefore, both the increase in H₂O₂, which inhibits CAT, and the decrease in NADPH, secondary to the decrease in G6PD, compromise CAT activity [20], which explains the significant decrease in CAT enzyme activity in the current acne patients. Thus, the significant decrease in CAT levels reflects the oxidative stress status in acne patients imposed by the oxidant-antioxidant imbalance.

SOD is thought to be one of the major enzymes that protects cells from ROS. It catalyzes the dismutation of superoxide radical (O₂⁻) to H₂O₂ [6]. Abdel Fattah et al. [3] found significant decrease in SOD activity in patients with severe acne in comparison with those with mild and moderate acne. This result is consistent with the present study in which we found decreased activity of SOD in the acne patients; however, unlike that reported by Abdel Fattah et al. [3], this did not correlate with disease severity. There is piling data that production of ROS by keratinocytes occurs upon exposure to toxic compounds such as inorganic arsenic [23] or ultraviolet radiation [24], there is as well a proof that purified tuberculin has been shown to activate TLR-2 on keratinocytes leading to production of ROS during tuberculosis infection [25]. These piled information implicate that whatever the mechanism involved in the induction of skin inflammation by P. acnes, ROS are most probably part of this process.

Grange et al. [2] found that P. acnes-stimulated keratinocytes produce superoxide anions (O₂⁻) and other ROS, which are involved in keratinocyte lysis, through their combination with nitric oxide to form peroxynitrates. They suggested that retinoic acid derivatives, which are the most effective antiacne drugs, act by preventing superoxide anion production, IL-8 release, and keratinocyte apoptosis.

The increased production of superoxide anion radicals (O₂⁻) seen in acne patients, increases the state of oxidative stress. SOD dismutates these dangerous radicals, reducing them into H₂O₂, which is further acted upon by CAT and GSH-PX, reducing them to water and oxygen. This oxidant–antioxidant system works in an efficient way regularly; yet, in a state of increased oxidative stress burden, as that taking place in acne patients, the rapid accumulation of H₂O₂ may deplete CAT enzyme too fast to handle the ROS that are produced abundantly in the cell [17]. Naini et al. [8] in their work concluded that CAT enzyme gets deactivated because of the high level of H₂O₂ released as part of the imbalance between the oxidative stress and the antioxidant enzyme systems. In this study, plasma MDA levels were significantly higher in the acne patients group compared with the healthy controls. This can be interpreted as a proof of oxidative stress as MDA is a by-product of lipid peroxidation. Similar results were found by Arican et al. [5], who detected significantly higher levels of MDA in acne patients’ plasma. Moftah et al. [15] reported significantly higher levels of MDA in the lesions of acne patients compared with controls. They also found a high statistically significant positive correlation between tissue MDA levels in acne lesions and the severity of these lesions. We found no correlation between MDA levels and age of patients, nor between MDA levels and blood antioxidant enzymes G6PD, CAT, and SOD levels. Similar results were reported by Arican et al. [5], who did not find any significant correlation between MDA levels and patients’ age, sex, or disease severity. Nielson et al. [26] also did not find significant relation between MDA levels and patients’ sex.

The high levels of MDA in acne vulgaris patients might be a direct reflection of the oxidative burden imposed by ROS released by inflamed damaged follicular walls. Furthermore, ROS also cause the release of chemotactic factors leading to neutrophil accumulation and activation, which perpetuates the inflammatory process and the release of lysosomal enzymes, which adds to the damage of the follicular epithelia [2].

The significant lower levels of antioxidant enzymes in acne patients do reflect a chronic systemic and local oxidative stress burden, which in turn leads to cell injury and progression of inflammation. The shift in oxidant–antioxidant balance might be due to the increased sebum production known to occur in acne patients (1.59-fold increase) [27], accompanied by the marked increase in squalene quantity (upregulated by 2.2-folds) [21]. In the absence of an efficient antioxidant system, the fat-rich sebum and squalene, which scavenge free radicals, get peroxidized inducing production of inflammatory mediators by skin keratinocytes and neutrophils [2]. Thus, the peroxide-damaged sebum, together with the state of inflammation, set the stage for the P. acnes to colonize sebum, which became a more suitable environment for this bacteria to start their own inflammatory insult on the skin [28]. Therefore, oxidative stress is definitely an important contributing cause of acne vulgaris, and its effect starts early in the disease. As P. acnes colonization takes place, oxidative stress becomes even worse, leading to a vicious cycle, hence chronicity.

The fact that no significant correlation was detected between any of the three antioxidant enzymes and disease severity, as well as between them and MDA plasma levels, in our acne patients might reflect the controversial results sometimes met when measuring oxidative stress using a limited number of antioxidant markers. Some studies reported a significant increase in SOD levels in acne patients compared with controls [5],
whereas others reported a significant decrease in the same enzyme [18]. Arican et al. [5] thought that the SOD-CAT system is affected differently in erythrocytes and leukocytes. Different results are also sometimes reached when measuring the same antioxidant enzyme such as GSH-PX in erythrocytes and in plasma [29,30]. The values in plasma can be modified in the course of obtaining the sample as a result of oxidation of molecules by the endogenous enzyme present in plasma despite the necessary conservative precautions taken to minimize this occurrence. Hence, the validity of some oxidative stress markers in plasma such as reduced GSH, thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation measured as MDA equivalents, has been criticized [31,32]. MDA was found to be increased in acne patients in some studies [5,15] and normal in others [18]. This study is in agreement with that of Arican et al. [5], who found no significant correlation between MDA levels and acne severity, duration, patient’s age, or sex, as well as between MDA levels and that of the other antioxidant enzymes. Active smoking, intense exercise, and overexposure to the sun are other causes of inaccurate results [33]. Some studies have suggested that the ratio of SOD/(GPX+CAT) activities in erythrocytes, rather than the absolute amounts of individual antioxidant enzymes, is a better indicative of oxidative imbalance [34].

Romeu et al. [33] found that if each biomarker is considered individually, no clear distinction is made between patients and controls, especially in relation to some of the biomarkers such as SOD, CAT, and TBARS. A clear distinction between patients’ and controls’ oxidative stress levels were found when a multibiomarker scoring system was used.

Romeu et al. [33] created a scoring system of oxidative stress (SOS) that uses a wide number of antioxidant markers, enzymatic and nonenzymatic, the sum of which (SUM function) linked to a Microsoft Excel spreadsheet, gave more reliable quantitative measures of oxidative stress compared with each marker considered individually. The SOS included enzymatic antioxidant markers such as glutathione S-transferase (GST), SOD, CAT, GSH-PX, and glutathione reductase, as well as nonenzymatic antioxidant markers including GSH, oxidized glutathione (GSSH), GSSH/GSH, both in erythrocytes and plasma, as well as lipid peroxidation products such as TBARS in erythrocytes and plasma. In addition, they used the discriminant analysis to evaluate the ‘manual scoring’. The larger number of statistically significant correlations obtained indicates that assessing oxidative stress using a single or few biomarkers is unacceptable. On the basis of that we recommend to use a wide combination of markers for multitoxidative evaluation of the clinical status of patients with diseases associated with oxidative stress status and to continue to try to reach a refined reliable scoring system to measure the global antioxidant capacity and a satisfactory way to determine the total antioxidant potential in different body fluids and tissues by measuring the ability of a biological fluid to resist oxidation.

In conclusion, the present work supports the presence of oxidative stress status in cases of acne vulgaris. The resulting oxidant–antioxidant imbalance can be an important early factor in the pathogenesis of the disease. This justifies adding a topical and/or systemic antioxidant to the treatment of acne vulgaris and calls for finding an easier, more accurate, and reliable multibiomarker scoring system for evaluating oxidative stress.

Acknowledgements
Conflicts of interest

There are no conflicts of interest.

References


