Study of oxidative stress in different clinical severities of acne vulgaris

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Background
Acne vulgaris is a multifactorial disease, but recent studies have focused on the role of oxygen free radicals and antioxidant enzymes. Malondialdehyde (MDA) is the end product of lipid peroxidation and is a good marker of free radical-mediated damage and oxidative stress. Superoxide dismutase (SOD) represents the major cellular defense against superoxide anions.

Objective
The objective of this study was to study the role of oxidative stress in acne vulgaris and to detect a possible link with the different clinical severities.

Patients and methods
Fifty patients with acne vulgaris and 20 healthy controls were included in this study. The severity of the disease was assessed using the Global Acne Grading System. The levels of SOD in erythrocytes and MDA in plasma were measured using a spectrophotometer.

Results
Although higher SOD levels and mean values were present in patients, there was no statistically significant difference compared with the controls. MDA levels showed a significant difference between patients and controls ($P < 0.05$), with MDA being higher in patients, indicating a condition of oxidative stress that had resulted from a high level of lipid peroxidation in acne patients. Comparison of SOD levels in patients showed that patients with severe acne had the lowest levels in comparison with patients with mild and moderate acne ($P < 0.001$). SOD levels were the highest in patients with mild acne. In terms of MDA levels, patients with severe acne showed the highest plasma MDA levels compared with those with mild and moderate ($P < 0.05$) acne, suggesting an increase in reactive oxygen species production overwhelming the antioxidant capacity. The lowest MDA levels were observed in mild acne.

Conclusion
Oxidative stress may play a role in the etiopathogenesis of acne and/or the progression of the disease. Coadministration of antioxidant drugs with various lines of treatment of acne might be helpful, especially for those with inflammatory lesions.

Keywords:
acne, malondialdehyde, oxidative stress, superoxide dismutase

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Introduction

Acne vulgaris is a common skin disease affecting more than 85% of adolescents and often continuing into adulthood [1]. Acne comprises lesions of various morphology, from comedones, papules, and pustules to nodules and cysts [2]. The pathogenesis of acne vulgaris is multifactorial and involves four main pathways. These include excess sebum production, abnormal keratinization of the follicles, propionobacterium acnes colonization, and inflammation of the follicle and surrounding dermis [3].

Recent studies on the etiopathogenesis of acne vulgaris have focused on the role of oxygen free radicals and antioxidant enzymes [4–6]. Inadequate antioxidant protection and/or excess reactive oxygen species (ROS) production creates a condition known as oxidative stress, which contributes toward the development of cutaneous inflammatory diseases [7–9]. It has been reported that oxygen free radicals, which are generated by the neutrophils on the follicular wall to kill microorganisms, may cause cell damage at the site of inflammation [10–14]. Sebum composition is altered in acne and ROS produced by neutrophils are involved in the irritation and destruction of the follicular wall that is responsible for the inflammatory progression of acne [15]. Superoxide dismutase (SOD) is a group of metalloenzymes that scavenges superoxide radicals and reduces their toxicity. It is an antioxidant that dismutates the O_2- anion to form O_2 and H_2O_2 [16]. Malondialdehyde (MDA) is the end product of lipid peroxidation. If antioxidant enzymes become incapable of dealing with the oxidative damage, oxygen free radicals initiate lipid peroxidation in cell and organelle membranes [17–19]. MDA is a good marker of free radical-mediated damage and oxidative stress [20,21].

The aim of this work was to study the role of oxidative stress in different clinical severities of acne vulgaris.

Patients

Fifty patients with acne vulgaris and 20 healthy controls were included in this study. Patients were selected randomly from the dermatology outpatient clinic at Alexandria University Hospital. Controls were age-matched and sex-matched healthy volunteers. A written informed consent was obtained from all cases. The Faculty of Medicine Ethical Committee approved the study.

Exclusion criteria

(1) Concomitant dermatological and/or systemic diseases because free radicals are likely to be elevated in these cases.
(2) The use of topical or systemic treatment affecting free radical scavenging such as vitamins, antibiotics, and anti-inflammatory drugs for 1 month before blood collection.
(3) Smoking and alcohol abuse as both can induce oxidative stress.

Patients and methods

All patients were subjected to complete assessment of history on the onset, duration, and the course of the lesions. Type of lesions, their number, and their distribution were recorded. The severity of the disease in every patient was assessed using the Global Acne Grading System (GAGS) [22]. The GAGS score depends on the site of lesions and number of lesions in each site, and according to the score, acne vulgaris is subdivided into the following grades: mild: 1–18, moderate 19–30, severe 31–38, and very severe ≥39.

Determination of superoxide dismutase and malondialdehyde

The parameters of oxidative stress such as SOD and MDA were measured in erythrocytes and in the serum, respectively, using a spectrophotometer.

Procedure

Venous blood was collected in heparinized tubes. After centrifugation, plasma was kept for estimation of MDA and erythrocytes were washed with saline and lysed with distilled water; then, the hemolysate was used for the estimation of SOD.

Principle of the malondialdehyde colorimetric assay

Thiobarbituric acid reacts with MDA in an acidic medium at a temperature of 95°C for 30 min to form a thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm using a spectrophotometer. The results were expressed as μmol/l.

Principle of the superoxide dismutase colorimetric assay

This assay relies on the ability of the enzyme to inhibit the phenazinemethosulfate-mediated reduction of nitrobluetetrazolium dye. The results were expressed as U/g Hb.

Statistical analysis was carried out using the Microstat computer software (Ecosoft, Indianapolis, Indiana, USA). The *t*-test was used to compare continuous data and the χ^2-test to compare categorical data. A value of 5% was considered as the cut-off level for statistical significance.

Results

Demographic data

The present study included 50 patients with acne vulgaris: 26 men and 24 women, with a mean age of 20.2 ± 4.0 years and a mean disease duration of 3.0 ± 1.1 years. Patients were graded according to GAGS as follows: mild (17 patients), moderate (17 patients), and severe (16 patients). Both SOD and MDA levels showed no correlation with age, sex, disease distribution, or duration ($P > 0.05$) (Table 1).

Comparison of superoxide dismutase and malondialdehyde levels in patients and controls

SOD levels in the present study showed no significant difference between patients and controls, although higher mean values were present in patients, but they were statistically insignificant ($P > 0.05$). SOD levels in mild acne patients were significantly higher compared with the controls, whereas in patients with severe acne, SOD levels were significantly lower compared with the controls ($P < 0.05$). Meanwhile, MDA levels were statistically higher in the patient group compared with the control group ($P < 0.001$) (Table 2).

Table 1 Patients' demographic data

Personal characteristics	Total number (*n*) = 50 [*N* (%)]
Age categories (years)	
10 to <20	26 (52.0)
20 to <30	24 (48.0)
Sex	
Males	26 (52.0)
Females	24 (48.0)
Disease severity	
Mild	17 (34.0)
Moderate	17 (34.0)
Severe	16 (32.0)
Disease duration (years)	
<1	0 (0)
1–3	31 (62.0)
>3	19 (38.0)
Disease distribution	
Face	24 (48.0)
Face and trunk	26 (52.0)

Comparison between superoxide dismutase and malondialdehyde levels in different grades of severity of acne

Comparison of SOD levels in the patient group according to the severity grades of acne (mild, moderate, and severe) showed that there was a significant difference in SOD levels in the three grades, being lower in severe cases and higher in mild cases ($P < 0.001$) (Fig. 1). Meanwhile, comparison of MDA levels in different acne severity grades showed that there was a significant difference between the three grades. MDA levels were significantly lower in mild cases and higher in severe cases ($P < 0.001$) (Fig. 2) (Table 2).

Correlation between superoxide dismutase and malondialdehyde in acne patients

A strong negative correlation was found between SOD and MDA levels in acne patients. ($r = -0.964$, $P < 0.001$) (Fig. 3).

Correlation between superoxide dismutase and malondialdehyde in grades of acne severity

A negative correlation was found between SOD and MDA in mild, moderate, and severe cases, but the strongest negative correlation was in the severe group ($r = -0.713$, $P < 0.001$; $r = -0.878$, $P < 0.001$; and $r = -0.895$, $P < 0.001$, respectively).

Discussion

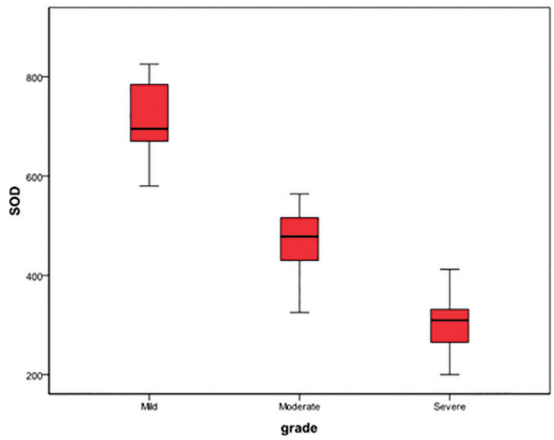
In the present study, SOD levels showed no significant difference in acne patients compared with the controls, although higher mean values were present in patients.

Table 2 Superoxide dismutase and malondialdehyde levels in all cases, grades of acne severity, and controls

Groups	SOD (U/g Hb)	MDA (μmol/l)
Cases ($n = 50$)		
Mean ± SD	496.5 ± 186.0	4.4 ± 1.9
Median (minimum–maximum)	489.0 (200–885)	5.0 (1.8–7.0)
Controls ($n = 20$)		
Mean ± SD	485.0 ± 61.7	1.4 ± 0.4
Median	487.5(350–850)	1.3 (0.6–2.1)
P value	$P > 0.05$**	$P < 0.001$**
Mild ($n = 17$)		
Mean ± SD	713.8 ± 87.9	2.1 ± 0.2
Median (minimum–maximum)	695 (580–825)	2.0 (1.8–2.7)
Moderate ($n = 17$)		
Mean ± SD	461.8 ± 73.7	5.0 ± 1.1
Median (minimum–maximum)	478 (325–564)	5.0 (3.2–6.8)
Severe ($n = 16$)		
Mean ± SD	302.7 ± 63.1	6.2 ± 0.6
Median (minimum–maximum)	309.5 (200–412)	6.2 (4.9–7.0)
P value	$P < 0.001$*	$P < 0.001$*

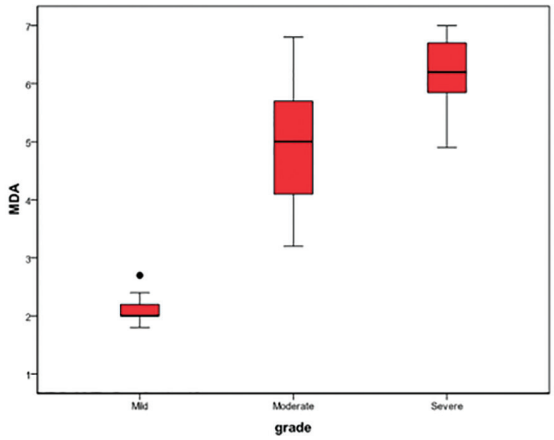
MDA, malondialdehyde; SOD, superoxide dismutase; *P value between mild, moderate, and severe acne grades; **P value between total acne patients and control.

Figure 1



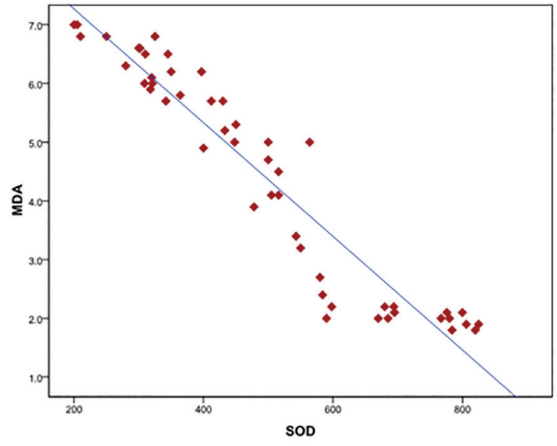
Comparison between superoxide dismutase (SOD) (U/g Hb) levels in the three grades of acne severity ($P < 0.001$).

Figure 2



Comparison between malondialdehyde (MDA) (μmol/l) levels in the three grades of acne severity ($P < 0.001$).

Figure 3



Correlation between superoxide dismutase (SOD) and malondialdehyde (MDA) levels in acne patients.

This is in agreement with Arican *et al*. [17] and Abdel Fattah *et al*. [21], who suggested that SOD levels increased as a reaction to oxidative stress occurring as a result of oxidant/antioxidant imbalance in the cells.

In contrast, Basak *et al.* [18] as well as Kurutas *et al.* [19] reported significantly low SOD activity in leukocytes obtained from patients with acne as compared with controls. According to Kurutas *et al.* [19], the decreased activity of SOD in polymorphonuclear leukocytes may be responsible for the increased levels of superoxide anion radicals causing epidermal damage and inflammatory changes in acne vulgaris. Meanwhile, Basak *et al.* [18] believed that the increase in ROS because of acne might be responsible for the decrease in SOD levels: singlet oxygen and peroxyl radicals can inhibit SOD activity.

Also, Sarici *et al.* [23] reported that SOD activities were significantly lower in the serum samples of the patients than that in the serum samples of the control group. They suggested that in acne vulgaris, the antioxidant defense system is damaged.

In the present study, we found that acne patients had significantly higher levels of MDA than the controls. This is in agreement with Arican *et al.* [17] and Sarici *et al.* [23], who suggested that this increase indicates the high levels of lipid peroxidation because of exposure to ROS. In contrast, Basak *et al.* [18] found insignificantly higher levels in the patient group. They attributed their results to the fact that the enzymatic antioxidant system was not completely inhibited. Thus, ROS generated by acne inflammation might be partially scavenged by the rest of the antioxidant defense system and might not cause significantly increased lipid peroxidation, even though some antioxidant enzymes were inhibited. The increase in SOD activities in patients with mild acne suggests that an adaptive response occurs to protect the cells from the hazardous products of free radical reactions, and reflects an appropriate activity of the antioxidant defense system as a response to oxidative stress, thus maintaining the MDA levels within the normal range. With increased severity of the disease process, the protective mechanism becomes inadequate, resulting in increased production of ROS that have the potential to initiate the lipid peroxidation chain reaction, leading to peroxidation of membrane lipids and other tissue lipids. This is shown with the decreased SOD activities and increased MDA levels observed in patients with moderate acne. In patients with severe acne, SOD activities were significantly decreased compared with mild and moderate cases and MDA levels were significantly increased, indicating an increase in ROS production overwhelming the antioxidant capacity.

In the present study, a statistically significant negative correlation was found between SOD and MDA. Also, negative correlations were found between SOD and MDA levels in the three acne severity grades cases. The strongest negative correlation was found in the severe group. Our results are in agreement with those of Abdel Fattah *et al.* [21], who assessed the oxidant /antioxidant system at both tissue and blood levels in patients with acne vulgaris with different acne severity grades. No correlation could be detected by Arican *et al.* [17], Basak *et al.* [18], and Kurutas *et al.* [19] between the severity of acne and the levels of SOD and MDA. According to them, this proved that oxidant/antioxidant balance may be affected to a specific extent in every patient, but the enzyme levels could not be influenced by the total amount of factors or agents associated with each case. Al-Shobaili *et al.* [24] suggested that markers of oxidative/nitrosative stress (MDA, NO, and SDO) might be useful in evaluating the progression of acne and in elucidating the mechanisms of disease pathogenesis.

Whether oxidative stress is a causative contributor or a mere consequence of acne is still controversial. The specific lipid that appears to be overproduced in acne is squalene. Higher levels of squalene peroxides and reduced vitamin E are noted in the sebum of acne patients. Squalene peroxides reduce the important skin antioxidant glutathione and generates ROS from neutrophils. The oxidation of sebum alters oxygen tension in the follicle, creating the ideal microaerophilic environment for the growth of propionobacterium acne. Propionobacterium acne does appear to promote inflammation in acne through the production of ROS. Upon keratinocyte exposure to propionobacterium acne surface proteins, immediate generation of ROS occurs [25].

Glutathione was lower in samples removed from acne-prone facial areas and in uninvolved areas sampled from the medial side of the upper arm of acne patients [26]. The same group of researchers has quantified lipid peroxides and inflammatory markers in comedone samples, the results providing further support that lipid peroxidation might be responsible for the progression of acne. Examination of comedo samples indicates that lipid peroxidation is evident in the earliest microcomedo and increases four-fold as inflamed lesions appear [27].

Conclusion

Oxidative stress may play a role in the etiopathogenesis of acne and /or in the progression of the disease. The antioxidant defense system is particularly damaged in cases of severe acne. However, it is still uncertain

whether oxidative stress is a cause or an outcome of inflammation. Coadministration of antioxidant drugs with various lines of treatment of acne might be helpful in treating acne patients, especially those with inflammatory acne lesions. Control studies are needed to observe the effect of various antioxidants on different grades of acne severity.

Acknowledgements

Conflicts of interest

None declared.

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