Pro-Inflammatory Factors And Oxidative Stress In Driving The Acne Process

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Abstract

Acne vulgaris is a chronic inflammation of pilosebaceous follicles with an unknown aetiology. Using the standard acne abnormalities as a starting point, the impact of free radical oxidation in acne vulgaris has received relatively few research. Therefore, this study was aimed to investigate the level of particularly pro-inflammatory substance which might derive and consequence acne and also examine the Antioxidants activity and their role in the regulation of oxidative stress in acne patients.

Material and method: This study was included a case control study. acne cases were divided into subgroups based on disease severity. Serum biomarkers level were measured for the following parameter: serum SOD, CAT and LOX activity, MDA levels, which were performed using spectrophotometer Technique. The association between biochemical markers and disease severity was evaluated. The efficiency of the predicting value was assessed using receiver operating characteristic (ROC) curve.

Results: The mean levels of lipid peroxidative products MDA and LOX activity were higher in acne patients groups (128.25, 3.92 mg/dl) compared to control group (28.11, 0.66 mg/dl). While the activity of enzymetics antioxidant (SOD, CAT) were decrease significantly in acne group (48.06, 53.6 U/L) compared to control (87.25, 84.19 U/L). Patients with acne who have high carbohydrate, fat and dietary product in their daily diet were associated with the Alterations in this balance of oxidative and antioxidant. Receiver operating characteristics curves indicated that the diagnostic performance of the SOD activity U/ml, MDA Con.(mol/L) and LOX activity in acne patient groups exhibited a good predictive value for prognosis of acne.

Conclusion: Serum LOX and SOD may represent a clinically useful stratification tool that provides important insights in patients with acne. Therefore, the current study could
suggest monitoring oxidative stress and antioxidant levels as a good biomarker for prognosis of acne.

**Keywords**: acne vulgaris, malondialdehyde, Lipoxygenase Activity, oxidative stress, reactive oxygen species, superoxide dismutase

**Introduction**

Acne disease is an inflammatory condition of the pilosebaceous duct caused by four major factors which contribute to the pathogenesis of acne vulgaris included: hyperplasia of sebaceous glands and increased sebum secretion, hypercornification of pilosebaceous duct, abnormal colonization, particularly by P. acnes, and inflammation [1].

Recent studies on the aetiopathogenesis of acne vulgaris were focused on the role of oxygen free radicals and antioxidant enzymes. If antioxidant enzymes become incapable in oxidative damage, oxygen free radicals initiate lipid peroxidation in cell and organelle membranes [2]. Oxidative stress causes damages to all cellular components through attacks on lipids, proteins and DNAs. Among these injuries, lipid damage through oxidative stress induced lipid peroxidation is particularly relevant to acne. The chemical pathogenesis of acne was proposed based on oxidative breakdown of lipid in the skin which is not just a consequence of acne process. Lipid peroxides, products of lipid peroxidation, may function as a cause of acne or as an acnegenic agents or both. Supporting data for this lipid peroxidation hypothesis comes from a study showing that lipid peroxidation occurs in acne and site specific free radical damage and products of lipid peroxidation might be involved in the initiation of inflammation [3]. The composition of sebum changes in acne, and neutrophil-produced reactive oxygen species (ROS) are implicated in the irritation and destruction of the follicular wall, which is responsible for acne's inflammatory progression [4].

Since the etiology and pathogenesis of acne are not completely understood, and a single, primary cause has not been identified, Therefore, this study was aimed to review the background documents in the scientific literature and focus on particularly pro inflammatory substance which might derive and consequence of acne.
Method:

In an attempt to investigate the potential clinical benefit of an antioxidant in offsetting the effects of oxidative stress, this study was included a case control study for acne patients. The medical data of seventy samples (fifty as acne patients, twenty five as control) from Imam Hussein medical city/ Kerbala were collected. After oral consent was obtained, survey information and photographs were taken during screening. Patient and Physician Evaluation form was completed. The sociodemographic aspects of all patients were collected through the self-reported technique including age, gender, BMI, family history of acne, some social activities and most common influencing factors such as obesity, oily and mixed skin, irregular menstrual cycles, sweet food, greasy food, dairy products, smoking, the improper use of cosmetics, the poor quality of sleep and stress. Acne cases were divided into subgroups based on disease severity. Serum biomarkers level were measured for the following parameter: serum SOD, CAT and LOX activity, MDA levels, were performed using spectrophotometer Technique. The association between biochemical markers and disease severity was evaluated. The efficiency of the predicting value was assessed using receiver operating characteristic (ROC) curve.

Results and discussion

Demographic and clinical characteristics

The chemical pathogenesis of acne was proposed based on oxidative breakdown of lipid in the skin which is not just a consequence of acne process. Several scholars [5,6] have attempted to correlate the clinical presentation of acne with some biochemical markers in an attempt to investigate the literature and examine the particularly pro inflammatory substance which might derive and consequence acne. The clinical demographic characteristics and laboratory parameters of both patient groups and the healthy control group were summarized in Table (1). Table illustrated the mean age of participants which was within mean age group of (22) years old. Gender distribution among the studied groups were: 16% male, 84% female for patient group, while 24% male and 76% female for control group.
The patient group were divided into groups based on the Types of Acne.

The most type of Acne incidence were for whiteheads, blackheads and papules type with about 58%, 32% and 24% respectively, while 14%, 6% were for the less types acne as in pustules, cysts. Table (2) was also demonstrated the social activities which were correlated to the biochemical markers.
Table (1): Descriptive of the Demographic and laboratory characteristics of the study population

<table>
<thead>
<tr>
<th>Study Characteristic</th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic Characteristic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Mean Age (Years)</td>
<td>20.34</td>
<td>22.76</td>
</tr>
<tr>
<td>Gender (male/female) No.</td>
<td>(8/42)</td>
<td>(6/19)</td>
</tr>
<tr>
<td>BMI (Mean Kg/m²)</td>
<td>23.97</td>
<td>23.55</td>
</tr>
<tr>
<td>smoking stat (yes, No)</td>
<td>(3.47)</td>
<td>(0.50)</td>
</tr>
<tr>
<td>Biochemical Markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD ACTIVITY (Mean U/ml)</td>
<td>48.06</td>
<td>87.25</td>
</tr>
<tr>
<td>CAT ACTIVITY (Mean U/L)</td>
<td>53.63</td>
<td>98.19</td>
</tr>
<tr>
<td>MDA Con. (Mean mol/l)</td>
<td>54.23</td>
<td>30.21</td>
</tr>
<tr>
<td>LOX activity (Mean µmol of HPO/min)</td>
<td>31.76</td>
<td>23.48</td>
</tr>
</tbody>
</table>

Types of Acne

<table>
<thead>
<tr>
<th>whiteheads</th>
<th>blackheads</th>
<th>papules</th>
<th>pustules</th>
<th>cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>29/50</td>
<td>16/50</td>
<td>12/50</td>
<td>7/50</td>
<td>3/50</td>
</tr>
</tbody>
</table>

Table (2): Descriptive characteristics of some social activities of study group

<table>
<thead>
<tr>
<th>social activities of study group</th>
<th>Acne Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eat carbohydrates in abundance (yes, No)</td>
<td>(46,4)</td>
</tr>
<tr>
<td>Eat more fats (yes, No)</td>
<td>(40,10)</td>
</tr>
<tr>
<td>Playing sports (yes, No)</td>
<td>(23,27)</td>
</tr>
<tr>
<td>His inheritance in acne (yes, No)</td>
<td>(24,26)</td>
</tr>
<tr>
<td>Female adrenal hormones disorder (yes, No)</td>
<td>(19,23)</td>
</tr>
<tr>
<td>Stress (yes, No)</td>
<td>(35,15)</td>
</tr>
<tr>
<td>Abundant consumption of milk and milk products (yes, No)</td>
<td>(44,6)</td>
</tr>
<tr>
<td>Eat chocolate a lot (yes, No)</td>
<td>(38,12)</td>
</tr>
<tr>
<td>Exposure to chronic infections (yes, No)</td>
<td>(31,19)</td>
</tr>
<tr>
<td>The use of cosmetics a lot or of a poor quality (yes, No)</td>
<td>(15,35)</td>
</tr>
<tr>
<td>Polycystic ovaries for females (yes, No)</td>
<td>(3,39)</td>
</tr>
</tbody>
</table>
Figure (1 A&B), demonstrated the distribution of oxidative stress biomarkers and enzymetics antioxidant in acne patients compared to the healthy group. Both serum SOD and CAT activities were decrease significantly in acne group compared to healthy control group.

In contrast, the mean levels of the lipid peroxidative product MDA and LOX activity were higher in acne patients groups (128.25, 3.92 mg/dl) compared to control group (28.11, 0.66 mg/dl).

Interestingly, the levels of lipid peroxidation markers were found to be negatively correlated with the activities of CAT or SOD activity in the serum of acne patients, see figure (2 C&D)

Figure (1A): Distribution of serum activity of antioxidant enzymes (A)catalase, (B) superoxide dismutas activity in acne patients group compared to the healthy group

Figure (2): Distribution of serum activity of oxidative stress markers (C) MDA, (D) LOX activity in acne patients group compared to the healthy group.
Since the pathogenesis of this disease is multifactorial, many compelling evidence suggests that oxidative stress is involved in the onset of acne [7]. In acne breakouts, changes occur in the content of 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 [8]. 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 [9]. 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 [10].

The high plasma levels of MDA in our acne patients may be a result of cellular damage caused by ROS. Many factors could be result in these reactive species, increasing level of ROS could be attributed to reduced levels of antioxidant enzymes [11]. Toxic molecules and reactive oxygen species play a crucial part in the pathogenesis and severity of acne vulgaris and might cause release of the chemotactic factors. 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 serum level [12]. On the other hand, LOX forms a family of lipid-peroxidizing enzymes, which are involved in the generation of lipid mediators. LOX products have been implicated in the pathogenesis of inflammatory skin diseases with keratinocyte hyperproliferation, such as psoriasis and chronic atopic dermatitis, and LOX inhibitors have been suggested for their treatment [13].

The involvement of LOX in the differentiation of sebaceous glands and follicular keratinocytes and its association with the development of acne lesions is a rather new idea. Lox have a role in the stimulating pro-inflammatory mediatorsand (such as Leukotrienes which are a family of eicosanoid inflammatory mediators) and therefore implicated in the initiation of acne lesions [14]. Keratinocytes express LOX with increasing differentiation. Leukotrienes is constitutively expressed, 5-LOX seems to overtake the downstream arachidonic acid metabolism and to be responsible for the enhancement of the pathway activity in the sebaceous glands of acne.
patients. Research findings reported that the presence of inflammation is the critical link between acne and eicosanoids. Also indicated that the sebaceous gland seems to be the key tissue in this relationship. Lipid analysis of sebum has detected no free arachidonic acid, but arachidonic acid can be found esterified within cellular membrane lipids. Thus, small amounts of free endogenous [15] or exogenous arachidonic acid liberated from neighbouring burst cells may be utilized by human sebocytes as arachidonic acid pool in order to form Leukotriene B4 (LTB4) which is a lipid mediator synthesized through arachidonic acid (AA) metabolism and it can induce neutrophil activation and cytokine secretion [16].

Another expression of inflammation in sebaceous gland is the release of cytokines/chemokines. They are not only present in the area of sebaceous glands but are also produced by human sebocytes [17]. The early presence of cytokines in microcomedones [64], can be considered as the initial inflammatory event in acne [18]. IL-1α, which induces comedone-like formation in vitro, is also produced by human sebocytes under stress conditions and induces IL-8 generation.

IL-6 and IL-8 expression was enhanced in acne-involved sebaceous glands in tissue. Moreover, IL-6 and IL-8 were produced by sebocytes in vitro and were up-regulated in the presence of the LOX activators. These findings make it likely that IL-6/IL-8 induction in human sebocytes occurs as a result of 5-LOX activation [19]. LTB4 may not only induce IL-8 production by macrophages, neutrophils and lymphocytes attracted to the acne-involved sebaceous follicles and stimulate lymphocyte proliferation [20], but may also stimulate sebocytes to produce IL-6 and, in the presence of Ca^{2+}, IL-8. Hence, an endless inflammatory cycle can be initiated, whereas LTB4 trigger the generation of inflammatory mediators [21].

**Examination the dietary factors on the oxidative stress / antioxidants levels in acne patients**

Acne vulgaris might be improved by dietary factors that increase insulin sensitivity [22]. Based on the hypothesized that a low-glycemic index diet would improve facial acne severity and insulin sensitivity. Although dietary factors have long been considered unimportant, many studies were reported that dietary carbohydrates have been implicated in the etiology of acne [23]. Figure (3) demonstrated a high level of lipid-peroxidizing enzyme (LOX) and final product [23] of the process (MDA) in patients with acne who have high carbohydrate, fat and dietary

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product in their daily diet which associated with the Alterations in this balance of oxidative and antioxidant.

![Figure 3: Effect of dietary factors on the oxidative stress/antioxidants levels in acne patients](image)

In case of elevated glucose concentrations in the bloodstream, most cells might suffer from pronounced oxidative stress. Both direct damage by AGE generated by glycation and damage indirectly caused by ROS during hyperglycemia can trigger an inflammatory response. Other mechanisms are also involved, such the deleterious action of AGE on their receptor (RAGE), which results in the production of ROS [24]. There is an association between ROS and lipotoxicity lies in the fact that they are oxidized in mitochondria by β-oxidation. The overload into the mitochondria because of increased FFA levels leads to an incomplete FFA oxidation, which generates an increase in ROS generation and toxic lipid intermediates. It is important to highlight that excess lipids are harmful in the case of saturated FFA, whereas mono and polyunsaturated FFA frequently exert antilipotoxic effects. The most abundant saturated FFA found in plasma is palmitic acid, which has been demonstrated to induce oxidative stress through β-oxidation in mitochondria and other pathways [25]. On the other hand, the association between repeated inflammation and acne was also studied. Figure (4) indicated high levels of oxidative stress markers and low level of antioxidant activity in patient of acne compared to control group.
**Figure (4): Effect of repeated inflammation on the oxidative stress/antioxidants levels in acne patients**

In such case, the immune response is triggered by the invasion of immune cells such as neutrophils and macrophages. All these factors produce ROS and oxidative stress promoting the inflammatory status. Furthermore, ROS promote inflammation by enhancing the levels of proinflammatory cytokines and the expression of cellular adhesion molecules and growth factors [26].

**Receiver operating characteristics of oxidative stress/antioxidants levels in acne patients**

Receiver operating characteristics (ROC) curve analysis of oxidative stress makers and antioxidant was performed. The best area under the ROC curve (AUC) for the acne patients was for SOD ACTIVITY (AUC = 0.96, p < 0.001) Figure (5) . ROC analysis indicated that SOD ACTIVITY <12 U/ml was predictive of increasing oxidative stress at the expense of antioxidant with 98% sensitivity, 82% specificity as shown in Table (3).

**Table (3): Differentiation power (area under the ROC peak, Sensitivity % and Specificity %) of the SOD ACTIVITY (U/ml) in acne patients**

<table>
<thead>
<tr>
<th>SOD ACTIVITY U/ml</th>
<th>AUP</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Cut-off points</th>
<th>Asymptotic Sig</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.965</td>
<td>0.98</td>
<td>0.82</td>
<td>12</td>
<td>&gt;0.001</td>
<td>0.929-1.000</td>
<td></td>
</tr>
</tbody>
</table>
Figure (5): receiver operating characteristics (ROC) curve analysis of SOD

On the other hand, ROC analysis of the MDA demonstrated an area under the curve equal to (0.865) as shown in figure (6) when the MDA levels > 21.4(mol/L) in acne patients with 98% sensitivity, 76% specificity as indicated in Table (4).

Table (4): Differentiation power (Area under the ROC peak, Sensitivity % and Specificity %) of the MDA level (mol/l) in acne patients

<table>
<thead>
<tr>
<th>MDA Con.(mol/L)</th>
<th>AUP</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Cut-off points</th>
<th>Asymptotic Sig</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.865</td>
<td>0.98</td>
<td>0.76</td>
<td>21.4</td>
<td>&gt;0.001</td>
<td>0.783-0.946</td>
<td></td>
</tr>
</tbody>
</table>
Figure (6): Receiver operating characteristics (ROC) curve analysis of MDA.

Further, Figure (7) showed the ROC analysis of the LOX which demonstrated an area under the curve equal to (0.918) when the LOX levels > 20 (µmol of HPO/min) in acne patients with 92% sensitivity, 82% specificity (Table (5)).

Table (5): Differentiation power (Area under the ROC peak, Sensitivity % and Specificity %) of the LOX activity (µmol of HPO/min) in acne patients.

<table>
<thead>
<tr>
<th>LOX activity</th>
<th>AUP</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Cut-off points</th>
<th>Asymptotic Sig</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.918</td>
<td>0.92</td>
<td>0.82</td>
<td>20</td>
<td>&gt;0.001</td>
<td>0.853-0.983</td>
</tr>
</tbody>
</table>
Several reviews have suggested a role of oxidative stress in the pathophysiological pathway leading to adverse outcomes associated with acne. Indeed, the major finding of this study was that SOD ACTIVITY significantly decreased with acne patients and associated with increased ROS and lipotoxicity which results from increased LOX activity. The increased levels of pro-oxidative biomarkers (MDA) and decreased levels of antioxidant (SOD and CAT) were related to inflammatory process. Therefore, serum LOX and SOD may represent a clinically useful stratification tool that provides important insights in patients with acne. In conclusion view, since LOX catalyzes LTB\textsubscript{4} production, inhibition of LOX provides an attractive target for down-regulation of inflammatory processes in the sebaceous gland. In such case of acne patients, that could reduce the inflammatory lesions and also decrease the synthesis of sebum lipids, especially of pro-inflammatory. It might be also suppressed the process not only by regulating inflammation and interleukin release but also for lipid synthesis [14]. Therefore, the current study could suggest monitoring oxidative stress and antioxidant levels as a good biomarker for prognosis of acne.

\textbf{Figure (7): receiver operating characteristics (ROC) curve analysis LOX}
Final conclusion:
This study was clarified and investigated the effect of some risk factors in acne patients and correlated with oxidative stress biomarkers and enzymetics antioxidant activity. Results were indicated that both serum SOD and CAT activities were decrease significantly in acne group compared to healthy control group. In contrast, the mean levels of the lipid peroxidative product MDA and LOX activity were higher in acne patient groups. Serum LOX and SOD may represent a clinically useful stratification tool that provides important insights in patients with acne.

References:


