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Original Research

Oxidative stress in acne vulgaris: an important therapeutic target

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Abstract

Objective: There has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. The aim of this study is to investigate the existence of oxidative stress and inflammatory marker IL-8 in patients with acne vulgaris, and the role of oxidative stress as a therapeutic target in the treatment of acne vulgaris.

Methods: A randomized prospective clinical trial was carried out on 56 patients of both sexes with age range of 14-35 years who attend to outpatient clinic in Al-Hussein Teaching Hospital-Kerbalaa -Iraq over a period from December 2011 to May 2012, all patients examined clinically by dermatologist and classified according to disease severity. Serum levels of glutathione (GSH), malondialdehyde (MDA) and interleukine -8 (IL-8) in the acne patients were measured by using ready- for- use Elisa kits, and compared to that of 28 healthy volunteers.

Results: The results of the serum level analysis of MDA for the acne patients (expressed as the mean± standard deviation) was highly significant (P value ≤ 0.001) higher than that of healthy volunteers, while serum level of GSH was highly significant (P value ≤ 0.001) lower in acne patients compared to healthy volunteers; there is a significant difference (P value ≤ 0.05) found in serum levels of IL-8 between the acne patients and the healthy volunteers.

Conclusions: The results obtained in this study clearly showed the existence of oxidative stress in patients with acne vulgaris, and that oxidative stress along with inflammation play a critical role in acne pathogenesis; furthermore, oxidative stress in acne patients may represents a potential therapeutic target and interference with antioxidant is a rationale choice.

INTRODUCTION

Acne vulgaris is a common human skin disease. It is a disease of the pilosebaceous unit in the skin, characterized by areas of skin with seborrhea, comedones (blackheads and whiteheads) which are the non-inflammatory lesions; papules (pinheads) and pustules (pimples) which are the inflammatory lesions, nodules (large papules) and possibly scarring [1]. Acne is an exclusively human disease and a unique condition of human sebaceous follicles of the face, chest and back that begins in the prepubertal child. Spontaneous regression is common, but in about 5% of cases acne persists beyond the age of 25 and extends into the fourth and fifth decades of life [2].

The earlier the symptoms start, the more severe is the course of the disease. The prevalence of the disease does not reflect any preference for male or female, but usually the course is more severe in males [3]. Acne vulgaris is a distressing skin condition which can carry with it a significant psychological disability. Patients with acne are more likely to experience anger and are at increased risk of depression, anxiety, suicidal ideation [4].

Acne has a complex etiology, involving abnormal keratinisation, hormonal function, bacterial growth, and immune hypersensitivity. The disease is limited to pilosebaceous follicles of the head and upper trunk because the sebaceous glands in these regions are particularly active. The primary acne lesion is the "blackhead" (microcomedone), an impaction and distension of the follicle with improperly desquamated keratinocytes and sebum. The stimulus for comedogenesis is uncertain [5].

At puberty, when androgens stimulate the production of sebum, pre-existing comedones become filled with lipid and may enlarge to become visible. Subsequently, some patients also begin to show signs of inflammation. Inflammatory acne is the result of the host response to the follicular inhabitant *Propionibacterium acne*, which is a member of the normal flora, largely incapable of tissue invasion or serious infection. The organism metabolizes sebaceous triglycerides, consuming the glycerol fraction and discarding free fatty acids. As a consequence of growth and metabolism, *P acne* produces neutrophil chemoattractants. *P acne* also activates complement and is generally inflammatory when brought into contact with the immune system [6].

In recent years there has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. Emerging studies have shown that patients with acne are under increased cutaneous and systemic oxidative stress [7]. Recently there has been renewed interest in the influence of oxidative stress and the operations of the antioxidant defense system in acne. Many of these investigations have examined the extent to which a potential oxidative stress burden in the skin might be reflected in the blood of acne patients [8].

It has been demonstrated that sebum composition is altered in acne, and ROS production by neutrophils is involved in the irritation and destruction of the follicular wall and are thought to be responsible for the inflammatory progression of acne. In comedones, the proportion of linoleic acid to other lipids is markedly decreased. Linoleic acid was found to have inhibitory effects on the action of several types of ROS released by neutrophils (superoxide radical, hydrogen peroxide and hydroxyl radical). As a consequence, in comedo lesions, ROS become excessive due to a lack of scavengers. Among skin superficial lipids, squalene which is specific to human sebum, appears to act as a quencher of singlet oxygen in vitro protecting skin surface from lipid peroxidation; however, its oxidation generates squalene peroxides, which exert comedogenic effects. Squalene peroxide is also thought to be the link between comedogenesis and bacterial colonization. It has been shown that fluctuation in the

cell redox environment induced the gene expression of pro-inflammatory cytokines including IL-1, IL-6, TNFα, and IL-8 [9]. Based on the above findings, it would seem reasonable that clinical interventions with oral and topical agents designed to support the antioxidant defense system would be helpful in acne.

The aim of this study is to investigate the existence of oxidative stress represented by serum malondialdehyde (MDA), and antioxidant state represented by serum glutathione, in addition to inflammatory marker IL-8 in patients with acne vulgaris, in addition, the role of oxidative stress as a contributing factor in combination with inflammation in the pathogenesis, also the extent at which interference with antioxidant may be valid in the treatment of patients with acne vulgaris.

SUBJECTS AND METHODS

Subjects

A randomized prospective clinical trial was carried out on:

Group A: 56 patients of both sexes with age range of 14-35 years who attend to outpatient clinic in Al-Hussein Teaching Hospital-Kerbalaa -Iraq over a period from December 2011 to May 2012 and approved by scientific and ethical committee of hospital, all patients examined clinically by dermatologist and diagnosed as having acne vulgaris that determined as mild, moderate or severe, complete history was taken from each patient.

Inclusion Criteria: The selected patients were complaining of papulopustular acne, and had never taken previous acne therapy treatment, or stopped any systemic and topical treatment at least one month before starting the present study.

Exclusion Criteria: Patients with chronic diseases, diabetic patients, those who were taking steroids, hypercholestremic patients, and patients with hepatic and /or renal insufficiency were excluded from the study. Pregnant and lactating women were also excluded from the study.

Group B: 28 healthy people, of both sexes, with age matched group who serve as control group.

Each subject was told about the study, informed consent was obtained from each one.

Methods

Blood Collection and Serum Separation: The blood was collected from the patients as well as the healthy volunteers by using a plastic 5 ml syringe, a blood sample of five milliliters was collected from each patient by vein puncture, left to stand for 15 minutes in a gel containing tube (the gel enhances blood

components to separate faster), then centrifugation would be done for 15-20 minutes at a speed of 4000 rounds per minute. An automatic pipette was utilized to move the sera from the test tubes to the Eppendorf tubes (dividing each serum sample into three parts, each has a volume of 0.5 ml or more) were the samples would be kept and frozen.

Laboratory Parameters

Serum levels of glutathione (GSH), MDA and IL-8 in the acne patients as well as healthy volunteers were measured by using ready- for- use Elisa kits of the mentioned parameters. The results obtained would be compared with those of the healthy people to find out if there was any difference between the acne patients and the healthy people.

GSH assay

The microtiter plate provided in Elisa kit has been pre-coated with an antibody specific to GSH. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for GSH and avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain GSH, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of GSH in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve [10].

MDA assay

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to MDA has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with HRP-conjugated MDA and incubated. A competitive inhibition reaction is launched between MDA (Standards or samples) and HRP-conjugated MDA, the less antibody bound by HRP-conjugated MDA. Then the substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of MDA in the sample. The color development is stopped and the intensity of the color is measured [11].

IL-8 assay

The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-8 Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-8 and avidin conjugated to horse- radish peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain IL-8, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm±2 nm. The concentration of IL-8 in the samples is then determined by comparing the O.D. of the samples to the standard curve [12].

Statistical analysis

Utilizing SPSS software package, student t-test was done, P value ≤ 0.05 considered significant change, all results represented as mean± SD.

RESULTS

Healthy volunteers were subjected to testing their serum levels of GSH, MDA and IL-8. The results were (expressed as the mean± standard deviation): 1.61+0.99 mcg/ml for GSH, 5.46+2.82 mcg/ml for MDA, and 36.65+25.73 pg/ml for IL-8. The results of the serum level analysis of GSH, MDA and IL-8 for the acne patients were: 0.65+0.22 mcg/ml for GSH, 8.68+1.55 mcg/ml for MDA and 61.17+39.92 pg/ml for IL-8. Statistical analysis by the use of unpaired t-test revealed that there was a highly significant difference (P value ≤ 0.001) found in serum levels of GSH, MDA, and a significant difference (P value ≤ 0.05) found in serum levels of IL-8 between the acne patients and the healthy people, (Fig.1, 2).
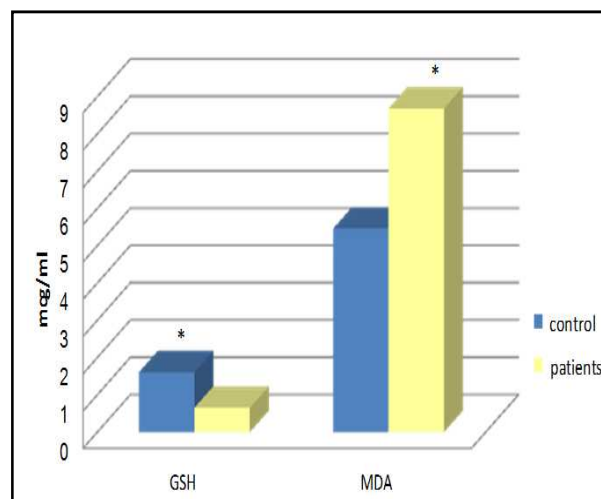

Fig 1. Comparison of serum GSH and MDA concentration between healthy and patients groups
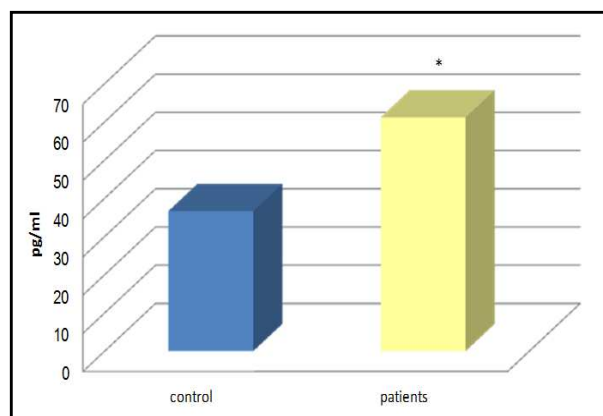
Fig 2. Comparison of serum IL-8 concentration between healthy and patients groups

DISCUSSION

Acne is a multifactorial skin disorder with multiple mechanisms involved in its pathogenesis; despite the great advances in dermatology, the precise sequence of events that involve in acne pathogenesis is still unclear [13]. Acne is characterized by sebum overproduction, follicular hyperkeratinization and an increased release of inflammatory mediators. Androgens, microbes and other pathogenetic influences are also at work in the development of acne [14]. Furthermore, oxidative stress within the pilosebaceous unit consider another important initiating step in the pathogenesis of acne [15]; taken together, inflammation and oxidative stress -both local and systemic- might set the stage for all subsequent events that lead to acne; beside the complex interaction between inflammation and oxidative stress in acne.

The results obtained in this study clearly showed the increased level of both oxidative stress and inflammation in acne patients compared to healthy subjects; there is a highly significant P≤0.01 difference in the serum level of MDA which is the end product of lipid peroxidation in patients with acne compared to healthy subjects; at the same time, highly significant reduction in serum glutathione level in acne patients compared to healthy subjects, indicating the strong involvement of oxidative stress in acne process. On the other hand, the level of inflammatory marker IL-8 is highly significantly increased in patients with acne indicating the pivotal role of inflammation in acne.

It has been reported that blood antioxidant enzyme activities superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) of 52 patients with papulopustular acne were significantly lower than control [16]. In another study, there was evidence of increased oxidative stress as reflected by higher thiobarbituric acid reactive substance [17], separate investigation have found elevated MDA [18] a well known marker of oxidative stress. Emerging studies indicate that low blood SOD and GSH-Px with elevated level of MDA are characteristics of acne vs. healthy control [19]. Furthermore, it has been concluded that decline in antioxidant activity manifested by a decrease in GSH quantity may play an important role in pathogenesis of acne vulgaris [20]; all the above mentioned evidences may support the results obtained in this study concerning the existence of oxidative stress in patients with acne compared to control.

It has been well documented that ROS play critical roles in many of the inflammatory skin diseases [21]. *Propionibacteriun acnes* taking part in acne pathogenesis cause the release of some chemotactic factors leading to neutrophils accumulation, this cause damage to follicular epithelium after the release of some inflammatory factors such as lysosome enzymes as a result of phagocytosis. ROS released from active neutrophils in the inflammatory tissue. These oxidants attack membrane lipids and cause chemical damage to any molecule in front including normal tissue [22]. Squalene which is specific to human sebum protects skin surface from lipid peroxidation, the peroxidated squalene lead to comedogenic effects [23]. It has been that exposure of peroxidated squalene products to human keratinocyte cells stimulates production of inflammatory cytokines and up regulates lipoxygenase activity [24]. It is clearly shown that leukotriene B4 implicated in promoting inflammation in acne even in the absence of *Propionibacteriun acnes*; leukotriene B4 is a chemoattractant capable of recruiting ROS-generating neutrophils, and its inhibition has been shown to improve acne in clinical practice [25]. These findings clearly showed the strong interaction between inflammation and oxidative stress and their involvement in the pathogenesis of acne vulgaris.

It has been found that one particular ROS, superoxide anion, was generated by epidermal cells following *Propionibacteriun acnes* stimulation and this process is associated with production of soluble inflammatory molecule IL-8 [26] a result that orchestrate with that obtained in this study.

Based on these findings, it is rationale to use agents that decrease level of superoxide anion such as antioxidants that act as free radical scavengers, or using agents that support the antioxidant enzymes SOD and GSH-Px, and through this, both oxidative stress and inflammation will be reduced. On the other hand, when antibiotic has been used to treat *Propionibacteriun acnes*, it is better to choose antibacterial agent with antioxidant activity and thus hitting more than one bird by one stone, antioxidant effect of drugs such as erythromycin, tetracycline and metronidazole which are used in acne treatment were very good choice and are preferable to the other antibacterials [27].

In conclusion, the results obtained in this study clearly showed the existence of oxidative stress in patients with acne vulgaris, and that oxidative stress along with inflammation plays a critical role in acne pathogenesis; furthermore, oxidative stress in acne patients may represents a potential therapeutic target and interference with antioxidant is a rationale choice.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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