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Pro-Inflammatory factors and oxidative Stress In triggering acne

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Supervisors Certification

We certify that this M.Sc. thesis titled:-

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Was prepared under our supervision in laboratories at department of Chemistry and Biochemistry- College of Medicine/ University of Kerbala as a partial requirement for **the Master Degree in Clinical Chemistry**

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Dedication

To …..

" My Father" and to "My Mother" who always encouraged me to reach Success.

My husband, brother, sister, and my daughter for their true feelings and to the Researchers and students

I dedicate this work.

MARWA

بِـــــسْمِ اللهِ الرّحْـمنِ الرَّحِيـــــمِ

وَلَقَـــدْ جَعَلْنَا فِـــــــي السَّمَاءِ

بُرُوجًا وَزَيَّنَّاهَا لِلنَّاظِرِينَ

صَدَقَ الله ألعلي العظيم

سورة الحجر: الآية (16)

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summary

Acne vulgaris is a chronic inflammation of pilosebaceous follicles with multi factorial disorder. Four major factors contribute to cause of acne vulgaris: hyperplasia of sebaceous glands and increased sebum secretion, hypercornification of pilosebaceous duct, bacterial infection , particularly by P.acnes(Propionibacterium granulosum), and inflammation. Recent studies on the aetiopathogenesis of acne vulgaris were focused on the role of oxygen free radicals and antioxidant enzymes. when antioxidant enzymes become incapable in oxidative damage, oxygen free radicals initiate lipid peroxidation in cell and organelle membranes. Oxidative stress causes damages to all cellular components through attacks on lipids, proteins and DNA. Among these effects , 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. 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 come 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. Since the etiology and pathogenesis of acne are not completely understood, and a single, primary cause has not been identified, Therefore, this study 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.

This study included acne. collectionThe medical data of 75 samples (50 acne patients and 25 control) from Imam Hussein medical city in a Kerbala –Iraq(during the period from January to march 2021)were collected.Acne cases were divided into

subgroups based on disease severity. Serum biomarkers level were measured for the following parameter: serum SOD, CAT , LOX activity and 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.

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.

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List of Abbreviation

Abbreviations	Full nomenclature
µl	Microliter
µmol/ml	Micromole/Milliliter
13-HPODE	13-hydroperoxyoctadecadienoic acid
4-HNE	4-hydroxyalkenal
5-LOX	5-lipoxygenase
AP-1	Activating protein-1
AUC	Area Under the Curve
BMI	Body mass index
Ca^{+2}	Calcium ion
CAT	Catalase
Cu	Copper
CuZnSOD	COPPER,ZINC-SUPEROXIDE DISMUTASE.
CVD	Cardiovascular disease
dH_2O	Double distilled water
DNA	Deoxy Ribonucleic Acid
E2	Estradiol
EC-SOD	Extracellular SOD
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
ERO1	Endoplasmic reticulum oxidoreductin 1
Fe	Iron
Gm	Gram
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
HEL	Hexanoyl-Lysine
HIF-1α	Hypoxia-inducible factor 1-alpha
HO^*	Hydroxyl radical
HPO	High Performance Organization
IGF-1	Insulin-like growth factor 1
IL-1α	Interleukin 1 alpha
IL-8 g	Interleukin 8
Interleukin-6	Interleukin-6
IR	infrared rays

kDa	Kilodaltons
kg/m^2	Kilogram / square meter
KH_2PO_4	Potassium Phosphate Monobasic
KU	Kilounits
KU/L	kilounits per liter
LDL	low-density lipoprotein
LOX	Lipoxygenase
LTB4	Leukotriene B4
MDA	Malondialdehyde
MetS	Metabolic Syndrome
Mg	Milligram
mg/dl	milligrams (mg) per decilitre
Ml	Milliliter
mM	Millimetre
mmol/l	Millimole/Liter
Mn	Manganese
MnSOD	Manganese superoxide dismutase
mTORC1	Mechanistic target of rapamycin complex 1
Na_2HPO_4	Sodium hydrogen phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nm	Nanometer
NO_2	Nitrogen dioxide
NOS	Nitric oxide synthase
NOX	Nitrous Oxide
Nr-f2	Nuclear factor-erythroid factor 2-related factor 2
O^*	Superoxide anion
O2	Oxygen
oxLDL	Oxidized low-density lipoprotein
P53	Protein p53
PCOS	Polycystic ovary syndrome
PH	Potential of hydrogen,
PPARs	Peroxisome proliferators-activated receptors
PPAR-γ	Peroxisome proliferator activated receptor-γ
PUFA	Polyunsaturated fatty acid
ROC	Receiver operating characteristics
ROS	Reactive oxygen species

Se	Selenium
SGs	Sebaceous glands
SO_2	Sulfur dioxide
SOD	Superoxide dismutase
SREBP-1	Sterol regulatory element- binding protein 1
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
U/L	Units per litre
u/ml	Units / millilitre
U/ml	Units per millilitre
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet radiation
W/V	Weight/value
WHO	World Health Organization
Zn	Zinc
Zn-SOD	ZINC-SUPEROXIDE DISMUTASE
B-catenin	Beta-catenin
μM	Micromiter

Chapter One

Introduction & Literature Review

1. Introduction

1.1. General introduction

Acne vulgaris is a chronic inflammation of pilosebaceous follicles multi factorial disorder. Four major factors contribute to the pathogenesis of acne vulgaris: hyperplasia of sebaceous glands that increased sebum secretion, hypercornification of pilosebaceous duct, abnormal colonization, particularly by P. acnes (Propionibacterium granulosum) ,and inflammation **[1,2]** .

Abnormal epithelial desquamation and follicular obstruction might lead to the primary precursor lesion in acne, it might also promotes sebum production, causing these obstructed follicles to fill with lipid rich material and form visible open and closed comedones. Sebum serves as a substrate for bacterial growth, leading to proliferation of P acnes. Finally, P acnes releases chemical mediators that promote inflammation, which is propagated by traumatic rupture of comedones into the surrounding dermis.This inflammation manifests through the development of inflammatory papules, pustules, nodules, and cysts **[1,2] ,** as shown in figure (1.1) .

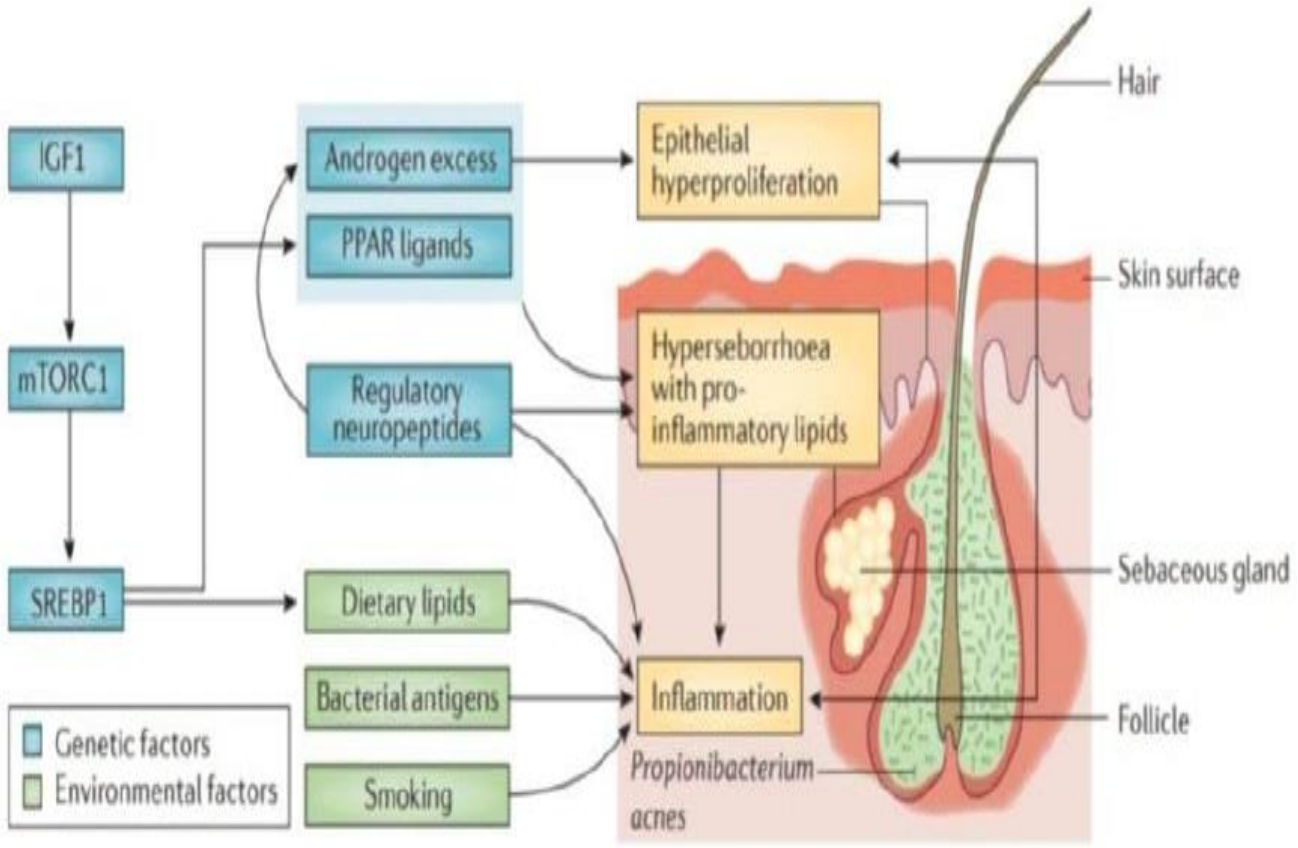


Figure (1.1) The network of four core events in acne formation [3] .

the function of the sebaceous gland is to excrete sebum. Sebum.is a mixture of relatively non-polar lipids, most of which are synthesized de novo by the sebaceous glands. The composition of sebum is remarkably species and age specific **[4,5]** .

Human sebaceous glands secrete a lipid mixture containing squalene and wax esters, as well as cholesterol esters, triglycerides and possibly some free cholesterol. For a long time hyperseborrhoea has been considered as a major aetiopathogenetic factor for acne. However, emerging data on alterations of sebum lipid composition in acne patients indicate that sebum composition may be more important for the development of acne lesions,than the secreted amount. Indeed, bacterial hydrolases convert some of the triglycerides to free fatty acids on the skin surface **[4,5]** .

On the other hand, there is also evidence that sebaceous glands can also synthesize considerable amounts of free fatty acids **[4,5]** . Although it is not directly life-threatening,acne vulgaris well documented that its severe as forms might profoundly impair quality of,life of the patients and, through social stigmatization, can lead to secondary psychological disorders **[4,5]** .

Despite extensive research efforts of the past decades, delicate details of the pathogenesis are still not completely unveiled. It is well known that acne is a multifactorial disease.Development and worsening of the symptoms can be triggered by psychological stress, alterations in the hormonal and nutritional status, etc **[4,5]**. It is widely accepted that the first step of the pathogenesis is the increase in the sebum production, and alteration of its composition **[6]** .

Such alterations (e.g. desaturation of fatty acids, presence of lipoperoxides) as well as the decrease of the,vitamin E content of the sebum were proven to trigger infundibular hyperkeratinization leading to comedo formation. In the closed comedos, bacteria (e.g. various Propionibacterium acnes strains) can over- grow, resulting in the acne-accompanying inflammatory processes of the sebaceous glands (SGs) **[6]** .

1.2. Chemical Pathogenesis of Acne

Acne affects the pilosebaceous units of the skin which presents with a variety of lesions at various inflammatory stages, including acne scars and hyperpigmentation **[7,8] .** According to Olutunmbi et al. **[7] ,** acne lesions are most commonly present on the face, chest, upper back and upper arms which are known to have a high density of sebaceous glands **[9] .**

The four main pathological factors involved in the development of acne are the increased sebum production, irregular follicular desquamation, Propionibacterium

acnes proliferation and inflammation of area **[10] .** These four factors are illustrated in figure (1.2) .

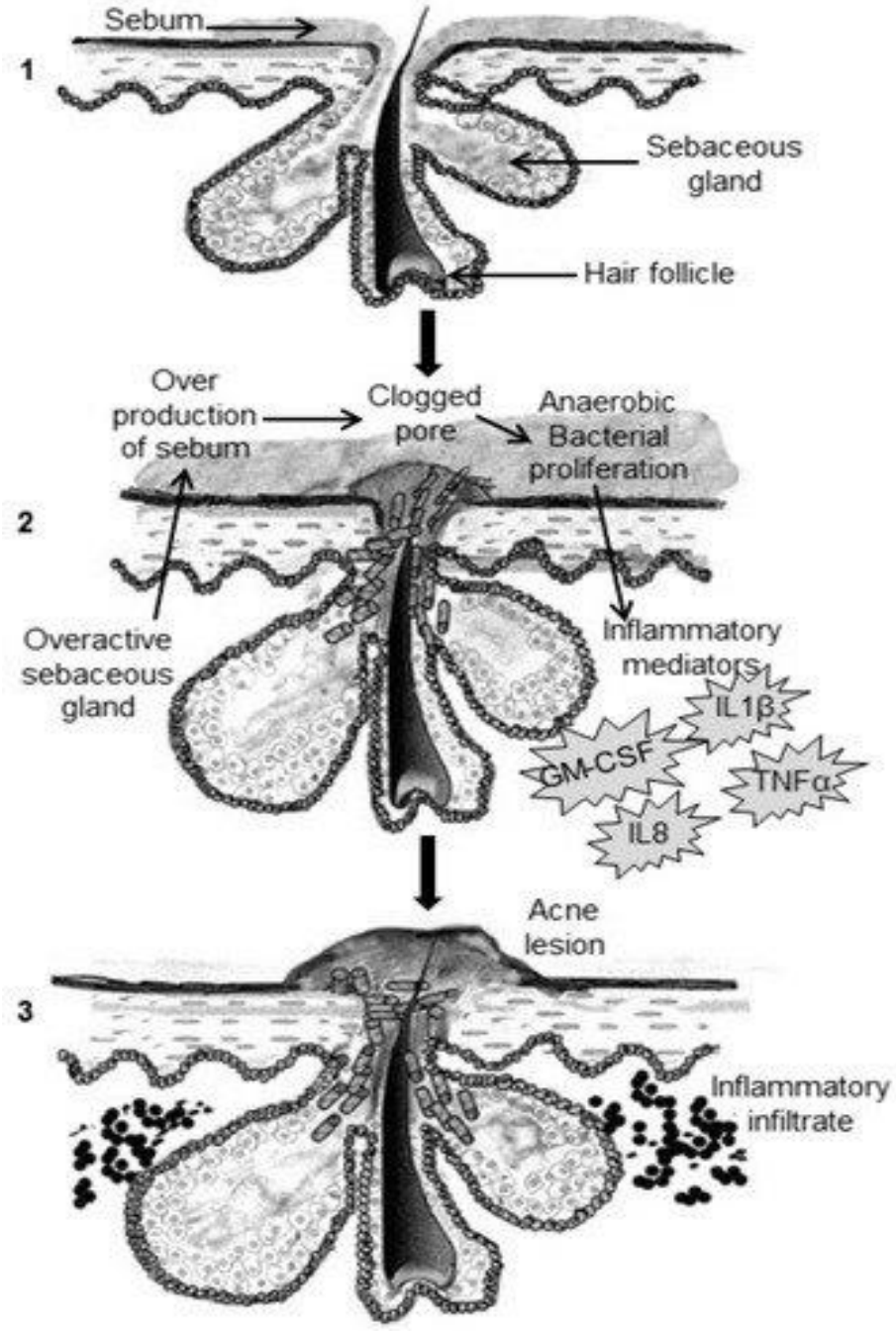


Figure (1.2) Contribution factors of the acne development of acne [10] .

1.2.1. Excess Sebum Production

Gollnick **[11]** stated that androgen hormones (especially testosterone) stimulate increased production and secretion of sebum. Increased sebum production directly correlates with the severity and occurrence of acne lesions and for this reason it is an important factor that should be taken into consideration when dealing with patients suffering from Acne vulgaris **[11,12] .**

1.2.2. Epidermal Hyper-Proliferation and Formation of Comedones

The keratinocytes in normal follicles are usually shed to the lumen as single cells which are then excreted. In patients with acne, hyper-proliferation of the keratinocytes occur and they are not shed as they should be , which results in the gathering of the abnormal desquamated corneocytes in the sebaceous follicle along with other lipids and monofilaments. This phenomenon results in comedogenesis **[10] .**

Webster **[13] ,** refers to a microcomedone as the first microscopic lesion that forms from occlusion of the follicle, and it is the precursor of the other acne lesions. The microcomedone gradually fills up with more lipids and monofilaments and develops into visible non-inflammatory comedones and inflammatory acne lesions **[10,11,14].**

Comedones are referred to as blackheads (open comedones) when they are dilated at the skin surface. They appear blackish on the skin and are filled with sebum and desquamated keratinocytes. They can also be termed as whiteheads (closed comedones) which appear as a white bump underneath the skins surface with no open pores. If sebum continues to accumulate, the closed comedone will continue to expand and may rupture into the surrounding tissue **[11] .**

1.2.3. Propionibacterium Acnes Infiltration (p.Acnes)

The microflora present in a normal sebaceous follicle is qualitatively similar to that found in comedones . This includes three coexisting groups of bacteria, namely (1) coagulase-negative staphylococci (Staphylococcus epidermidis) ; (2) anaerobic diphtheroids (P. acnes and Propionibacterium granulosum) and (3) lipophilic yeasts (Pityrosporum species) **[15] .**

P. acnes and S. epidermidis differ in their potential to provoke local skin inflammation and to generate pro-inflammatory mediators. It was however determined that S. epidermidis is not likely to partake in the pathogenesis of inflammatory Acne vulgaris skin lesions as the antibody response to S. epidermidis was somewhat harmless compared to the antibodies generated by P. acnes **[16] .**

As S. epidermidis is an aerobe organism and their growth site is superficial, it is incapable of residing in the anaerobe environment of the infra-infundibulum where the inflammatory process occurs. The lipophilic yeasts present in the pilosebaceous unit do not seem to play a noteworthy etiologic part in any disease conditions **[15].** P. acnes is an anaerobic, gram positive pathogen that colonizes in sebaceous follicles. It is generally more prevalent in areas of the skin that are densely packed with sebaceous follicles because these follicles produce large volumes of sebum that provide a lipid-rich, anaerobic environment that is optimal for P. acnes **[11] .** It is evident that all individuals have P. acnes present on the surface of the skin which can contribute to follicular clogging, but not all individuals present with acne due to the differences in individual immune response to the pathogen **[17] .** According to McInturff and Kim **[18] ,** P. acnes produces a lipase enzyme that metabolizes the triglycerides of sebum into glycerol and fatty acids, which may in turn assist in the formation of comedones and the inflammation that follows. P. acnes appears to be

the most probable organism to cause Acne vulgaris and is therefore the target of oral and topical antibiotic treatments **[15] .**

1.2.4. Inflammation Process

The inflammatory process begins when P. acnes is detected by the immune system. P. acnes has a highly inflammatory effect which may trigger the release of chemostatic factors such as lymphocytes , neutrophils and macrophages. These factors may cause follicular damage, rupture and leakage of bacteria, fatty acids and lipids into the surrounding dermis. This process will give rise to inflammatory lesions (pustules, nodules , cysts and papules) . Inflammatory lesions are filled with pus and are larger than non-inflammatory lesions **[8,10,11] .**

Additionally it was found that neutrophils generate reactive oxygen species (ROS) which partially contributes to acne inflammation by damaging the follicular epithelium. This leads to the expulsion of the follicular content into the dermis which consequently causes various inflammatory processes **[19] .**

1.3. Types of lesions that are common in acne vulgaris

1. **Papules – Red ,** inflamed bumps on the skin that feel tender and have no head are called papules. Squeezing a papule will not get rid of it faster and may cause scarring **[20,21] .**
2. **Whiteheads** –Whiteheads result from a pore that is blocked completely. The trapped oil, bacteria, and dead skin cells cause a white head to form on the skin's surface **[20,21] .**
3. **Blackheads –** When a pore is partially blocked, blackheads often form. The trapped bacteria,oil, and dead skin slowly drains to the surface of the skin to form a blackhead.The dark color is caused by melanin in the skin reacting

with oxygen. Blackheads typically take a longer time to clear than whiteheads **[20,21] .**

4. **Pustules –** Pustules are the most common type of acne lesion. They usually appear as an inflamed red circle with a center that is white or yellow. They can be popped at home, but acne sufferers shouldn't touch it with their bare hands and make sure that the material they are touching the skin with is sterile. Acne medications may be more effective after the pustule has been popped **[20,21] .**
5. **Nodules –** Severe acne often causes nodules. Acne nodules are hard bumps under the skin that may be large and last for months. Scarring is a common side effect of nodular acne **[20,21]** .

1.4. Aggressive skin agent

1.4.1. Pollution effect

There is an increasing evidence from epidemiological studies that air pollution may impact on healthy human skin and contribute to extrinsic skin aging . In addition , a growing number of reports suggest a link between different types of air pollutants, such as nitrogen dioxide (NO_2) , sulfur dioxide (SO_2) , and ground level ozone, and their role in the aggravation of preexisting skin diseases .For example , exposure to air pollution has been reported to be associated with an aggravation of eczema in children living in Munich and Germany . It is currently not known if a similar relationship exists between air pollution and acne vulgaris , i.e., an inflammatory skin disorder , which is characterized by increased sebum production , abnormal keratinization of the pilosebaceous duct , and an inflammatory reaction to acnes **[22]**.

Air pollution is a major problem of recent decades, which has a serious toxicological impact on human health and the environment . The sources of pollution

vary from small unit of cigarettes to large volume of emission from motor engines of automobiles and industrial activities **[22]** .

1.4.2. Solar radiation

It is classified based on wavelength. The wavelength of ultraviolet rays (UVR) is less than 400 nm . Most studies on UVR have indicated that it causes skin aging. Therefore , sunscreen should be applied to protect against both UVA and UVB . Unlike UVR, IR radiation can penetrate the epidermis, dermis, and subcutaneous tissue . The effects of IR radiation on the skin have received less attention than UVR **[23]** .

Visible light, which is recognized by the human eye , is a spectrum of electromagnetic radiation with wave- lengths between 400 and 700 nm. Although individuals are exposed to visible light during daytime , little is known regarding the effects of visible light on the skin **[23]** .

In acne case, the excessive sebum secretion blocks air circulation in the hairs to create an environment that is conducive to the growth of P. acnes , an anaerobic bacterium . Porphyrin is produced in acne, and when it is irradiated with blue light as a light-sensitive substance , it produces singlet oxygen , which interferes with the chemical metabolic reaction of acne **[23].**

1.4.3. Oxidative stress and free readical

Oxidative stress is caused by exposure to reactive oxygen intermediates , such as superoxide anion (O^{*}) , hydrogen peroxide (H_2O_2) , and hydroxyl radical (HO^{*}) , which can damage proteins, nucleic acids , and cell membranes. Increasing evidence suggests that the cumulative damage caused by reactive oxygen species contributes to numerous diseases **[24,25]** .

1.5. Risk factors of oxidative stress

1. Menopause is a risk factor for oxidative stress : It has previously been demonstrated that estrogens (estradiol, or E2) function as sexual hormones as well as antioxidant molecules, thereby counter balancing oxidative stress . Estrogens , mainly E2 , have been reported to lower vascular oxidative stress by modulating the expression and function of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases as well as antioxidant enzymes (SOD, GPx, catalase),providing protection against oxidative stress during the reproductive stage **[26,27]** .
2. Metabolic Syndrome (MetS) : Growing evidence suggests a dominant pathogenic role for oxidative stress , a dominant event in cellular damage and dysfunction , given its strong relationship with various MetS combinations and resulting clinical complications . In fact , it has been proposed that MetS-induced oxidative stress is an early event in all metabolic manifestations in view of the association of the end products of free radical-mediated , insulin resistance (IR) state , hyperlipidemia, and hypertension **[27,28]** .

Oxidative stress and impaired glucose metabolism/insulin resistance IR plays a crucial role in interlinking the various constituents of the MetS cluster and in fortifying the syndrome's evolution . Oxidative stress has been shown to be associated with IR, a key feature of MetS . This association is not surprising, given the insulin action of glucose metabolism and cascade signaling , the abnormalities of which contribute to MetS . Indeed , insulin insensitivity leads to increase circulating glucose levels. Persistence of hyperglycemia is thought to favor glucose autoxidation and NOS activation , which collectively enhance oxidative stress. Furthermore , elevated circulating and cellular oxidative stress markers in patients with nascent MetS are associated with increased levels of oxLDL , nitrotyrosine,

monocyte superoxide and NOX activity along with a decrease in the nuclear factor erythroid-derived 2 like 2 (Nrf_2) antioxidant defense system **[27]** .

3. Acne can be related to some endocrine diseases : the most common in females is polycystic ovary syndrome (PCOS) . There are acne symptoms in 70 % of PCOS cases. PCOS is typically characterized by hyperandrogenism, chronic anovulation , and polycystic ovaries . Women with PCOS have abnormalities in the metabolism of androgens and estrogen , and in the control of androgen production . PCOS is also associated with peripheral insulin resistance and hyperinsulinemia, and obesity amplifies the degree of both abnormalities **[28]** . An increase in oxidative stress-derived inflammation has been hypothesized to be a major mechanism in the pathogenesis and progression of obesity-related disorders . Additionally, a rise in inflammatory cytokine levels might drive a further increase in oxidative stress, setting up a vicious cycle . The complex and intimate association between both increased oxidative stress and increased inflammation , not only in obesity but also in related disorders such as type-2 diabetes and cardiovascular disease (CVD) , makes **[29]** .

1.6. Oxidative Stress and skin

Oxidative stress is an imbalance between oxidants (free radicals) and antioxidants in favor of free radicals . Cellular injuries or diseases are caused by the imbalance between production and removal of ROS in the skin . Many cellular processes , including cell metabolism , signaling pathways , inflammation , cell proliferation, and aging, are affected by oxidative stress. Increasing free radicals changes the structure and functions of the proteins , lipids and nucleic acids, and can lead to tissue damages **[30]** .

1.6.1. Oxidative stress and acne

Extensive research during last two decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation , which in turn could mediate most chronic diseases. Oxidative stress can activate a variety of transcription factors including NF-κB, AP-1, p53, HIF-1α , PPAR-γ, β-catenin/Wnt and Nrf_2 . Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules and anti-inflammatory molecules . It has been reported that the release of inflammatory factors is one of the earliest events to occur in the acne process . In addition, oxidative stress within the pilosebaceous unit alters the environment from one that is unsuitable to harbor anaerobic bacteria to one that is perfectly suited for the colonization of such species. Oxidation of sebum alters oxygen concentration in the follicle, which results in a favorable environment for P acnes to survive. Although further studies are needed, oxidative stress and inflammation set the stage for all subsequent pathogenic factors leading to acne **[30]** .

1.6.2. Reactive oxygen species and free radicals

Reactive oxygen species (ROS) are highly reactive molecules that are generated from oxygen metabolism . They can be free radicals or non-radicals . Free radicals are molecules that contain at least one unpaired valence electron at their outer shell, making them highly reactive and short lived **[31,32]** . radicals are reactive compounds that are naturally produced in the human body . They can exert positive effects (e.g. on the immune system) or negative effects (e.g. lipids, proteins or DNA oxidation) . Free radicals are toxic molecules, may be derived from oxygen , which are persistently produced and incessantly attack and damage molecules within cells

; most frequently.This damage is measured as peroxidized lipid products, protein carbonyl , and DNA breakage or fragmentation **[32,34]** . Collectively, the process of free radical damage to molecules is referred to as oxidative stress . To limit these harmful effects, an organism requires complex protection which is the antioxidant system. This system consists of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic antioxidants (e.g. vitamin E [tocopherol], vitamin A [retinol] , vitamin C [ascorbic acid] , glutathione and uric acid).Imbalance between free radical production and antioxidant defence leads to an oxidative stress state , which may be involved in aging processes and even in some pathology **[32,35]** . Sources of free radicals have two principle sources : endogenous sources and exogenous sources. Endogenous sources of free radicals include those that are generated intracellularly, acting within the cell, and those that are formed within the cell, but are released into the surrounding area. These intracellular free radicals result from auto-oxidation and consequent inactivation of small molecules such as reduced thiols and flavins . They may also occur as a result of the activity of certain oxidases, lipoxygenases,cyclo-oxygenases,dehydrogenases and peroxidases **[32,36]** .

Electron transfer from metals such as iron to oxygen-containing molecules can also initiate free radical reactions paradoxically ; antioxidants may also produce free radicals. Environmental sources of free radicals include exposure to ionizing radiation (from industry , sun exposure , cosmic rays , and medical X-rays) , ozone and nitrous oxide (primarily from automobile exhaust), heavy metals (such as mercury, cadmium, and lead), cigarette smoke (both active and passive) and other chemicals and compounds from food, water, and air **[32]** . The exogenous sources of free radicals resulting from ionizing radiation play a major role in free radical production **[32]** .

A number of studies indicate a strong role for increases in protein oxidation as a primary cause of cellular dysfunction observed during aging and age-related neurodegenerative diseases **[37]** . In acne, sebum produced by the 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 **[38]** .

Figure (1.3) shows the generation of ROS and its effects on cell function **[39]** , Also, Table (1.1 A & B) illustrated (B) the cellular sources of free radicals**[40]** , (A) the Exogenous and endogenous Sources of ROS **[41]** .

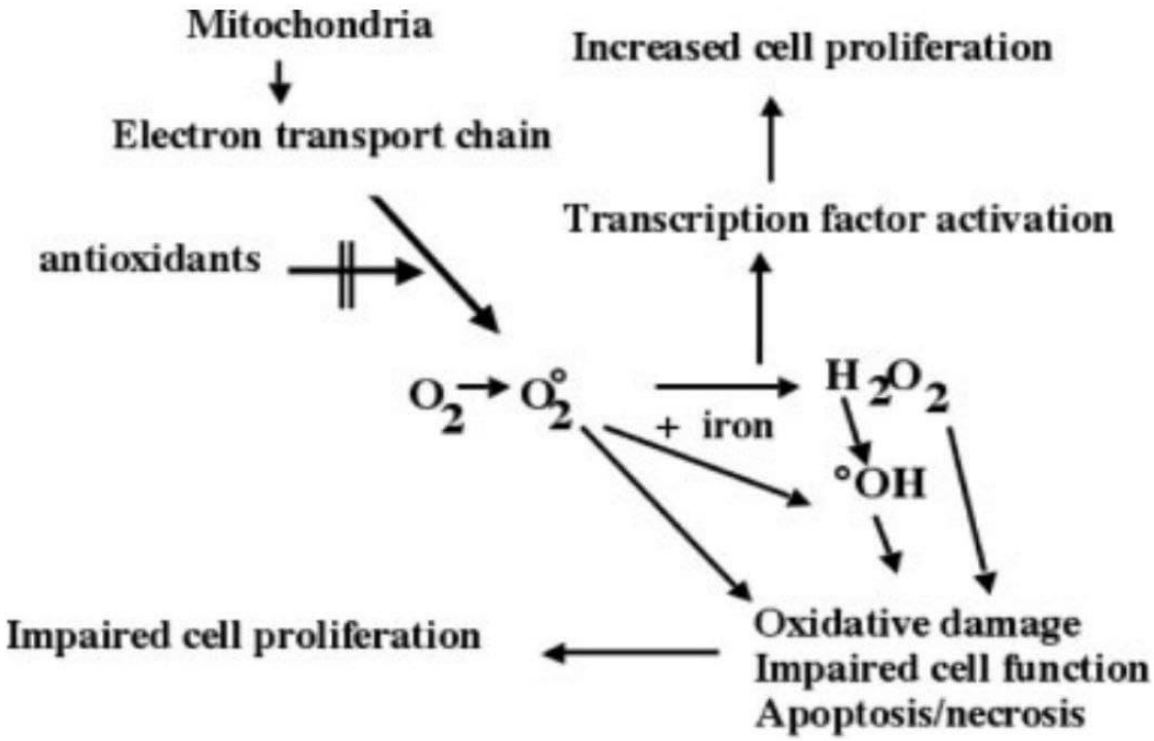


Figure (1.3) Generation of ROS and its effects on cell function [39] .

Generation of ROS and its effects on cell function . Through the electron transport chain , mitochondria process molecular oxygen. The superoxide ion (O2),

through superoxide dismutase , forms the more stable hydrogen peroxide H2O2 mole- cule. In the presence of iron or other metals such as copper, H_2O_2 can form the highly reactive hydroxyl radical (OH^-) . These radical species can be detoxified by antioxi- dants such as catalase . They can also result in various direct cellular effects such as oxidative stress and cellular dysfunction . Through signaling pathways involving redox sensitive transcription factors, these ROS can also result in cellular proliferation, differ- entiation, apoptosis, or necrosis **[39]** .

Table (1.1A) cellular sources of free radicasls [40] .

Organelles	Reaction
Endoplasmic Reticulum	cytochrome P450 dependent electron-transport
	cytochrome b5 dependent electron-transport
Mitochondria	electron transport (respiratory chain)
	lipid peroxidation
Lysosomes	myeloperoxidase system
Membranes	lipid peroxidation
	lipoxygenase prostaglandin synthase
	NADPH-oxidase
Peroxisomes	oxidases
	flavoproteins
Cytosol	hemoglobin
	oxidases
	transition metals

Table (1.1B) Sources of ROS and mediators of their catabolism [41] .

Exogenous sources of ROS	**Catabolism by antioxidant systems**
• Smoke	• Superoxide dismutases
• Air pollutants	• Catalases
• Ultraviolet radiation	• Glutathione peroxidases
• Y-irradiation	• Glutathione reductase
• Several drugs	• Thioredoxins
	• Thioredoxin reductases
Endogenous sources of ROS	• Methionine sulphoxide reductases
• NADPH oxidases	• Peroxiredoxins or peroxynitrite reductases
• Mitochondria	
• ER flavoenzyme ERO1	
• Xanthine oxidase	**Catabolism by small molecules that**
• Lipoxygenases	**react with ROS non-enzymatically**
• Cyclooxygenases	• Ascorbate
• Cytochrome P450 enzymes	•Pyruvate a-ketoglutarate
• Flavin-dependent demethylase	•Oxaloacetate
• Polyamine and amino acid oxidases Nitric oxide synthases	
• Free iron or copper ions	
• Haem groups	
• Metal storage proteins	

ER, endoplasmic reticulum: ROS, reactive oxygen species. Sources reviewed in REFS 10-12.

In acne, sebum produced by sebaceous glands, content a reactive oxygen species (ROS) which may be released from the impacted damaged of the 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 **[42]**.

1.7. Lipid peroxidation

Fundamentally, Lipid peroxidation is a ROS-involved forms in several toxic lipid aldehydes and lipid hydroperoxides which work as secondary signaling intermediates in reproduction of the oxidative stress signals which participate to the pathophysiology of human health and illness. Recent studies have demonstrated that the lipid peroxidation-derived lipid aldehydes regulate a number of human pathological complications including acne and various inflammatory diseases. Recent evidence also suggests that lipid peroxidation-derived lipid aldehydes act as biomarkers of various disease processes **[43]** .

lipid peroxidation products are also capable of inducing production of pro-inflammatory cytokines and activation of peroxisome proliferators-activated receptors (PPARs). PPARs are nuclear transcription factors involved in the control of lipid metabolism as well as in the control of inflammation **[44]** .

Mechanism of lipid peroxidation was involved in both polyunsaturated fatty acid (PUFA) and cholesterol which oxidized by enzymatic and non-enzymatic pathways [**45]** ,that is summurised in figure (1.4) .

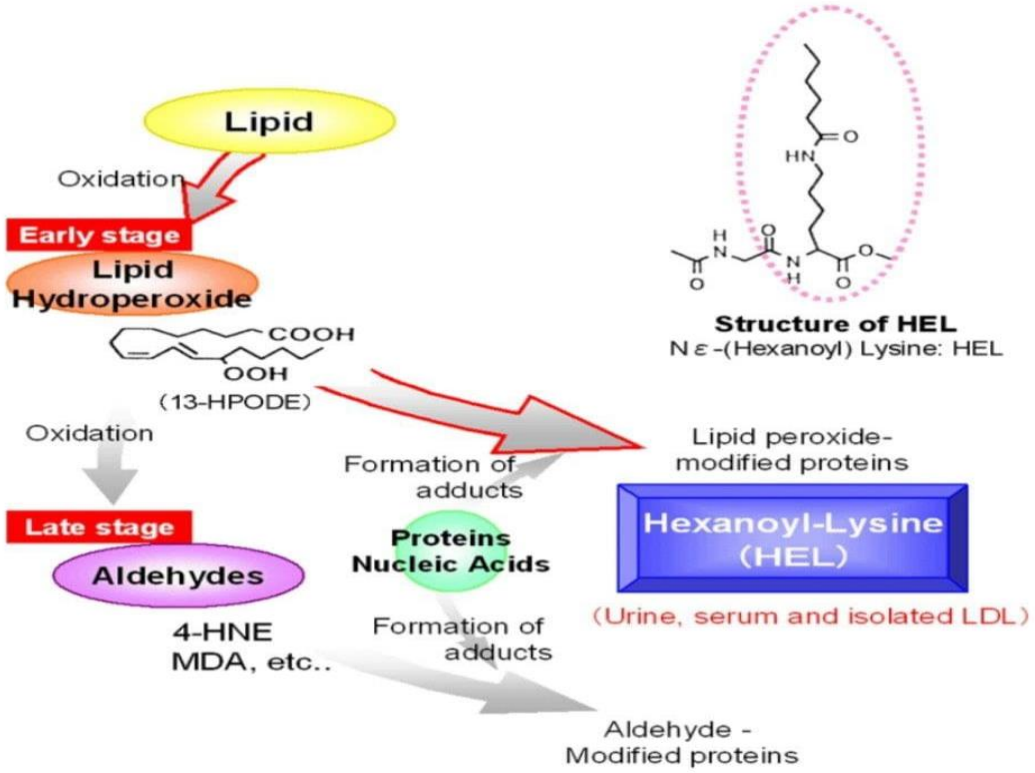


Figure (1.4) lipid peroxidation [33] .

1.7.1. Lipid peroxidation and acne

Lipid peroxidation is obvious in acne , and that localized free radical injury and peroxides might be included in the damage inflammatory processes , other studies determined that components of sebum, specifically squalene, display promoted comedogenicity when oxidized . Squalene was shown to be greatly sensitive to oxidation and authors showed that both squalene and its oxidized metabolites are presented at much greater concentrations in acne vs healthy control **[46]** .

Malondialdehyde, generated from the collapse of poly unsaturated fatty acids, acts as a suitable index for detecting the range of the peroxidation process . Malondialdehyde generation during the peroxidation of microsomal membranes differs among various forms of tissues , thus , it is hard to exactly compare the range of lipid peroxidation **[47]** .

Only unsaturated fatty acids with three or more methylene- intermittent double bonds can eventually produce malondialdehyde, so devirsty in this compound formation may be a reflux of the lipid structure rather than the capability to lipid peroxidation **[47]** .

Lipoperoxidation, on the other hand, has an effect on keratinocyte proliferation and differentiation . Additionally, lipid peroxidation products can also activate peroxisome proliferators-activated receptors and cause the generation of pro-inflammatory cytokines (PPARs) . PPARs are nuclear transcription factors that have a role in lipid metabolism as well as inflammatory regulation . Remarkably, enzymes implicated in the synthesis of several Eicosanoid metabolite products, such as 5-lipoxygenase (5-LOX), have been observed to be expressed at higher levels in acne-affected skin than in healthy skin **[48]** .

Inflammatory skin conditions characterized by keratinocyte hyperproliferation have been attributed to LOX products **[49,50]** . Induced IL-6 and IL-8 expression in human sebocytes are among the effects of 5-LOX activation **[51,52]** .

1.8. Antioxdant

Antioxidants are needed to prevent the formation and oppose the actions of reactive oxygen and nitrogen species , which are generated in vivo and cause damage to DNA,lipids , proteins , and other biomolecules . Endogenous antioxidant defenses (superoxide dismutases, H2O2-removing enzymes , metal binding proteins) are inadequate to prevent damage completely ,so dietderived antioxidants are important in maintaining health. Many dietary compounds have been suggested to be important antioxidants : The evidence for a key role of vitamins E and C is strong **[53] .**

1.8.1. Antioxidant Enzymes

The three major antioxidant enzymes - superoxide dismutase , glutathione peroxidase, and catalase which are unique in cofactor requirements and cellular locations **[54]** .

1-The superoxide dismutases

An enzyme convert superoxide anion into $H_2O_2$and O_2. All superoxide dismutases have at least one first transition series metal (Fe, Mn, or Cu) at the active site. CuZnSOD is found in plants, animals, and fungi **[54]** .In humans, there are three forms of SOD : cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) . SOD destroys Superoxide radical by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates **[55]** .

2. Catalase

Catalase is a tetrameric haemin- enzyme consisting of 4 identical tetrahedrally arranged subunits of 60 kDa .Therefore, it contains 4 ferriprotoporphyrin groups per molecule , and its molecular mass is about 240 kDa . Catalase is one of the most efficient enzymes known. It is so efficient that it cannot be saturated by H_2O_2 at any concentration. CAT reacts with H_2O_2 to form water and molecular oxygen **[56]** .

$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

3.Glutathione peroxidase :

It is a major protective system against endogenously and exogenously oxidative stress induced lipid peroxidation. The enzyme contains stoichiometric amounts of selenium and is reactive with a variety of organic hydroperoxides as well as with hydrogen peroxide **[57]** .

Two major types of glutathione peroxidase have been found. One type is distinguished by containing selenium in the form of covalently bound selenocysteine in its active site. This selenium-dependent enzyme is active with both organic hydroperoxides and H_20_2. The enzyme from bovine erythrocytes is a tetrameric protein of Mr – 80K **[58]** . The second type of glutathione peroxidase consists of proteins that do not depend on selenium for catalysis. This class is constituted by glutathione transferases, first described as proteins catalyzing the conjugation of GSH with electrophilic compounds such as aryl halides. These enzymes are dimeric proteins that often occur in multiple forms in the same organ, and some of the isoenzymes **[58]** .

1.8.2. Free Radical scavengers

The second line of defense inhibits the production of damaged species, apart from making the free radicals less harmful that further reduces damages caused by oxidative reaction. Some excellent scavengers of free radicals are vitamins E & C, flavonoids, and uric acid **[59]** .

Dietary antioxidants such as vitamin E, vitamin C, carotenoids, some minerals (e.g. Zn, Mn, Cu,Se) and polyphenols can affect the activity of endogenous antioxidants. Endo- and exogenous antioxidants may act synergistically to maintain or re-establish redox homeostasis **[60]** .

Vitamin E is the main lipid-soluble antioxidant in the body. As antioxidant, vitamin E acts in cell membranes where prevents the propagation of free radical reactions, although it has been also shown to have pro-oxidant activity. Non-radical oxidation products are formed by the reaction between u-tocopheryl radical and other free radicals, which are conjugated to glucuronic acid and excreted through the bile or urine **[61]** . The role of vitamin E in skin biology has not yet been illuminated. It is known to protect biological membranes from free radicals by antioxidant activity. Vitamin E has a role in immune system and metabolic processes **[62]** .

Antioxidative properties of vitamin E are generated in numerous physiological and pathological processes **[63]** .

Several compounds are known to function as water-soluble, chain-breaking antioxidants . For example, aseorbic acid, uric acid, cysteine, and glutathione scavenge oxygen-centred radicals and suppress the oxidation of lipids . The role of water-soluble chain-breaking antioxidants in the oxidations of lipids in aqueous dispersions can be most clearly understood by the use of water-soluble and lipid-soluble radical initiators, which generate radicals at a convenient, known and constant rate and at a specific site **[64]** .

Vitamin C is the major water-soluble antioxidant and acts as the first defense against free radicals in whole blood and plasma **[65]** .

Vitamin C has been shown to scavenge reactive oxygen species such as superoxide radical anion, singlet oxygen, hydrogen peroxide, and hydroxyl radical. These reactive free radicals are normally generated via biological metabolism of oxygen **[66]** .Ascorbic acid or vitamin C,it scavenges the generated radicals terminating inflammation accordingly **[67]** .

1.9. Knowledge gap

Acne vulgaris is known as a commonly-seen skin disease with a considerable impact on the quality of life. At present, there have been a growing number of epidemiological, medical, demographic and sociological researches focusing on various influencing factors in the occurrence of acne. Nevertheless, the correlation between biochemical factors and acne has yet to be fully investigated .

The etiology and pathogenesis of acne are not completely understood, and a single, primary cause has not been identified. In the past several decades, various studies have indicated that patients with acne are under increased cutaneous and systemic oxidative stress as indicated by observations of various oxidative stress biomarkers.This review is a part of case control study Which investigated the impacts of some biochemical markers on acne which construct a framework for the potential correlation between human biomarkers and patterns of acne by multidisciplinary research teams,since acne mostly occurs during adolescence, with age and gender playing a significant role in its occurrence. Besides, the prevalence of acne showed a decreasing trend with age. Males outnumbered females in terms of adolescence acne while it was the opposite in terms of post-adolescence acne. Moreover, acne can be affected negatively by such influencing factors such as family

history, overweight, 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. In addition, environmental factors play a crucial role, along with various natural environmental factors, including temperature, sun exposure and air pollution.

Therefore, a further qualitative and quantitative research is required to figure out the impact of most of these factors on acne. This study was attempt to determine the direct relationships between former factors and acne to cure such a chronic disease.

1.10. Research Aims and Objectives:

1-To evaluate the levels of lipidperoxidation and MDA as pro inflammatory factor of acne.

2- To examine the feasibility of employed SOD, CAT levels as a redox balance in inflammatory skin conditions.

3- To suggest areas where further research is needed, to deal with gaps in the knowledge related to role of antioxidants level in skin inflammatory prevention and treatment.

Chapter Two

Materials & Methods

2. Materials and methods

2.1. Study Design :

The present work included a case control study for a group of (75) samples: (50) patient samples, (25) healthy control samples. The study was conducted from January to July 2021.

patients with acne disease were selected from the withdraw blood unit Karbala, Al Hussein Teaching Medical City. History of family, smoking state, weights and heights were taken from each subject. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire) including age, gender, BMI, history of diseases, some social activities, hormones disorder, Exposure to chronic infections, the use of cosmetics of a poor quality and having some current chronic diseases. They were also exposed to medical examination for signs and symptoms of acne vulgaris by specialized doctor based on the World Health Organization (WHO) criteria.

2.2. Instruments:

In this chapter, materials, instruments and tools were described and listed in table (2.1).

Table (2.1): The instruments used in the study

No.	**Instruments**	**Suppliers**
1	Balance	Germany
2	Centerfuige	Sigma _Germany
3	Deep freezer	Hitachi (Japan)
4	Disposable Syringe	China
5	Eppendorf tube	China
6	Gel tube	China
7	micropipette	Salmed _Germany
8	spectrophotometer	japan
9	Test tube	China
10	Vortex mixer	Cyan (Belgium)
11	Water bath	Karlkole_Germany

Materials

12	2 - thiobarbituric acid	Germany
13	6.Ammonium molybdote tetrahydrate	Germany
14	Hydrogen peroxide	Germany
15	Pyrogallol	Germany
16	Sodium hydroxide	Germany
17	Trichloro acetic acid	Germany
18	Tris buffer	India

2.3. Inclusion and Exclusion criteria:

Patients Criteria:

All patients were subjected to the full clinical history, clinical examination, and relevant laboratory investigations. The diagnosis of the acne vulgaris clinical conditions was established according to the latest clinical practice guidelines by the WHO. The type of acne was identified based on evaluation of laboratory measurements for the clinical assessment of acne vulgaris .

Patients Exclusion criteria : No medical condition has been ruled out.

Control Criteria:

Control group of an apparently healthy 25 subjects (6 male and 19 female) were chosen from well-known volunteers participants. Blood samples were drawn from the volunteers, participants had no history of acne vulgaris diseases. The percentage of female and male adult individuals were about the same in the patients frame. The ages of the participants were also convergent in the whole study group. Demographic information of the participants were also collected through the self- reported technique (student questionnaire).

2.4. Study variables :

Dependent Variable : SOD, CAT and LOX activity, MDA levels

Independent Variable: Age, gender, BMI, and some social activities

Approval of the Ethical Committee

The protocol of the study was approved by Ethical Committee of Kerbala Medical College, and committee of dermatology consultant unit in Al Hussein Teaching Medical City. Samples from serum were obtained after consent from patients or the patients' relatives .

2.5. Measurement and Data collection

Data Collection

A structured questionnaire was specifically design to obtain information which helps to select individuals according to the selection criteria of the study. Sociodemographic aspects of the subjects (patients and control) were also collected through the self-reported technique (student questionnaire) .

Blood Collection and Storage

Blood samples were collected from Draw blood unit of Al Hussein Teaching Hospital. Five mls of blood samples were drown by venipuncture using 5ml disposable syringes, blood was left for (15 min) at room temperature in gel tube. Serums were separated by centrifuging for 10 minutes at approximately 4000 xg . Serum samples were aliquot into four eppendrof and store at -20°C to avoiding multiple freezing-thawing cycles and used for the further measurement. Blood collection tubes were be disposable, non-pyrogenic, and non-endotoxin.

2.6. Methods:

2.6.1. Determination of Body Mass Index:

The body mass index (BMI) was calculated by the following equation:

$$\boldsymbol{BMI}=\mathbf{W}\boldsymbol{eight}\ (\boldsymbol{kg})/\mathbf{H}\boldsymbol{eight}^{2}\ \textbf{(meters)}$$

The body mass index (BMI) is the metric currently in use for defining anthropometric height/weight characteristics in adults and for classifying (categorizing) them into groups as shown in table (2.2**) [68] .**

Table (2.2): Body mass index.

Weight status	BMI range (kg/m^2)
Underweight	15-19.9
Normal weight	20-24.9
Overweight	25-29.9
Class I obesity	30-34.9
Class II obesity	35-39.9
Class III obesity	≥ 40

2.6.2. Measurement of serum Catalase activity by using spectrophotometer Technique:

Principle :

Catalase activity was assessed by incubating the enzyme sample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37 °C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374 nm against the blank **[69]** .

Samples and Reagents Preparation:

1. Sodium, potassium phosphate buffer (50 mM, pH 7.4): this buffer is prepared by dissolving 1.1 g of Na_2HPO_4, and 0.27 g of KH_2PO_4 in 100 ml distilled water.

2. H_2O_2 (20 mM) in 50 mmol/l sodium, potassium phosphate buffer: this solution is freshly diluted and standardized daily using a molar extinction coefficient of 43.6 $M^{-1} cm^{-1}$ at 240 nm.

3. Ammonium molybdate (32.4 mmol/L) **.**

Instrument: Shimadzu 1800 spectrophotometer was used in the study. Procedure shown in table (2.3) .

Table (2.3) Samples and Reagents Preparation

Reagents	Test	Control-test*	Standard	Blank
Serum	100 µl	100 µl	–	–
D.W.	–	1000 µl	100 µl	1100 µl
Hydrogen peroxide	1000 µl	–	1000 µl	–
Mix with vortex and incubate at 37 °C for 3 min, after that, add:				
Ammonium molybdate	4000 µl	4000 µl	4000 µl	4000 µl
After that, the tubes were kept at room temperature. Changes in absorbance were recorded at 374 nm against the reagent blank.				

Assay procedure:

Catalase activity was assessed by incubating the enzymes ample in 1.0 ml substrate (65 m mol/ml hydrogen peroxide in 60 m mol/l sodium-potassium phosphate buffer, pH7.4) at 37 °C for three minutes. Their action was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at374nm against the blank .

Calculation :

The rate constant of a first-order reaction (k) equation is used to determine catalase activity

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * \left[\log \frac{S^{\circ}}{S - M}\right] * \frac{Vt}{Vs}$$

t: time.

S◦: absorbance of standard tube.

S: absorbance of a test tube.

M: absorbance of control test (correction factor).

Vt: total volume of reagents in test tube.

Vs: volume of serum.

2.6.3. Measurement of serum Lipoxygenase enzyme activity by using spectrophotometer Technique:

Principle :

This method determines the increase on absorbance at 234 nm as a result of the formation of conjugate double bonds in the linoleic acid hydroperoxide **[70]** .

Samples and Reagents Preparation:

1. Sodium, potassium phosphate buffer (50mM, pH 6.4): this buffer is prepared by dissolving 1.1g of Na_2HPO_4 and 0.27g of KH_2PO_4 in 100ml distilled water.

2. Linoleic acid as a substrate: it is prepared by dissolving 70 mg of linoleic acid and equal weight of tween 20 was dissolved in 4 ml of deionized water and followed by sufficient amount of 0.5 N sodium hydroxide was added to a clear solution and then made up to 25 ml using deionized water **.**

Assay procedure:

The typical reaction mixture contains 2.8 ml of 50mM sodium phosphate buffer pH 6.4. 20 µl of serum and the reaction was initiats by addition of substrate to the reaction mixture, maintained to have 250µM for linoleic acid in the total volume. One unit of enzyme activity is defined as the amount of micro moles of hydroperoxide formed per minute **.**

Calculation:

The lipoxygenase activity is calculated by using the following formula:

LOX µmol of HPO/min activity =(Δabs*Total volume of the mixture reaction)/(0.00275*volume of the sample)

2.6.4. Measurement of serum Superoxide dismutase by using spectrophotometer Technique:

Principle :

The principle of this method is based on the production of pyrogallol-quinone through a reactive intermediate, the semiquinone radical, and the ability of SOD to inhibit this reaction by radical dismutation. Pyrogallol-quinone is brown and absorbs visible light at 420 nm **[71] .**

Samples and Reagents Preparation:

-Tris buffer (pH 8.0): was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylenediaminetetraacetic acid (EDTA) in dH_2O and completing the volume to 100ml. .

- Pyragallol solution (0.2 mM): was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH_2O .

Assay procedure:

According to Marklund and Marklund (1974), reaction mixture consists of 50 µl of serum with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH_2O. As a blank , dH_2O was used. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation .

Calculation

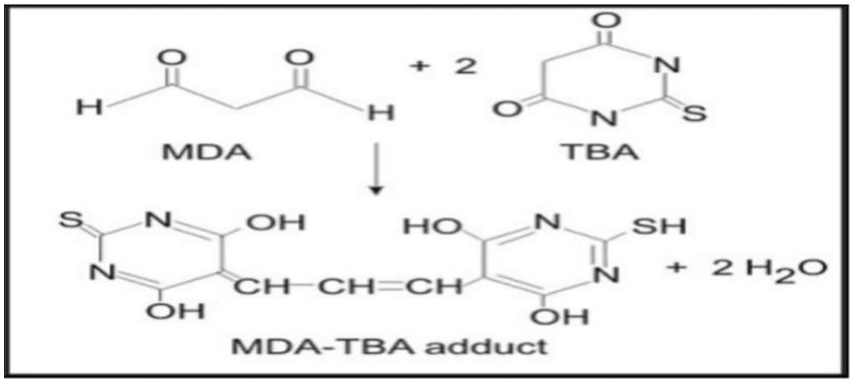
- SOD activity was calculated using the following equation:
- SOD activity (u/ml)= (Vp-Vs)/(Vp*0.5)*(Vt/Vs)*n
- Vp=Auto oxidation rate of pyrogallol (control)

- Vs= Auto oxidation rate of sample (with enzyme)
- Vt=Total reaction volume (ml)
- Vs= volume of enzyme used for the assay (ml)
- n= dilution fold of the SOD sample
- 0.5= factor for 50% inhibition

2.6.5. Measurement of serum Malondialdehyde by using spectrophotometer Technique:

Principle :

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. The basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm **[72] .**



Samples and Reagents Preparation:

Stock TCA – TBA – HCl Reagen

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in

100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl **.**

Assay procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in a boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink color was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml, distilled water, and 0.6 ml TCA-TBA-HCl reagent were mixed and boiled. Blank was always taken .

Calculation :

the extinction coefficient of MDA at 535 nm is = 1.56 × 105

MDA concentration = χ / 0.0624 nmol / ml

2.7. Statistical Analysis:

Information from the questionnaire , all test results from patients and control samples were entered a data sheet .

The data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copy-right (2013 – 2020) .

Descriptive statistics was performed on the participants' data of each group. Values were presented by n (%) for categorical variables. The distribution of the data was checked for normality using the Box plot test .

ANOVA test was used to adjust other risk factors including: age, gender (male, female), BMI. The 95% confidence intervals (95%CI) were also determined for all variables .

Significant differences in continuous variables among the parameters were con- firmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant. Receiver operating characteristics (ROC) curves was also used to test the markers' diagnostic performance in both and control groups .

Chapter Three

Results & Discussion

2. Results and discussion

3.1. Demographic and clinical characteristics

Acne vulgaris is a chronic inflammation of pilosebaceous follicles with an unknown aetiology. It is the most common skin disorder and may be a sign of bad social activities and quality of life **[46, 73]** .

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 **[46, 73]** 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 of acne.

Also, this study was brought the light to the knowledge gap regarding the potential therapeutic target of Antioxidants in the regulation of oxidative stress of acne patients. The clinical demographic characteristics and laboratory parameters of both patient groups and the healthy control group were summarized in table (3.1).

Table illustrated the mean age of participants which was within mean age group of (22) years old. Gender distribution among the studied groups were: 8 male, 42 female for patient group, while 6 male and 19 female for control group.

The patient group were divided into groups based on the types of Acne. The descriptive table also shown an adjustment of other risk factors which were collected through the self-reported technique (student questionnaire), these factors included: BMI, history of diseases, some social activities and many others.

The most types of Acne lesions incidence were to be for whiteheads , blackheads and papules type with about 29, 16 and 12 respectively, while 7, 3 were for the less types acne as in pustules, cysts.

Most of patients that involved in this study have social activities which were correlated to the main biochemical markers and demonstrated in table (3.2) .

Table (3.1): Descriptive of the Demographic and laboratory characteristics of the study population .

Study Characteristic		**Patients**	**Control**
Demographic Characteristic	No. of patients	50	25
	Mean Age (Years)	20.34	22.76
	Gender (male/female) No.	(8/42)	(6/19)
	BMI (Mean Kg/m^2)	23.97	23.55
	smoking stat (yes ,No)	(3,47)	(0,50)
Biochemical Markers	SOD ACTIVITY (Mean U/ml)	48.06	87.25
	CAT ACTIVITY (Mean U/L)	53.63	98.19
	MDA Con. (Mean mol/l)	54.23	30.21
	LOX activity (Mean µmol of HPO/min)	31.76	23.48

Types of Acne	**whiteheads**	**blackheads**	**papules**	**pustules**	**cysts**
NO.	29/50	16/50	12/50	7/50	3/50

Table (3.2): Descriptive characteristics of some social activities of study group

social activities of study group	Percent
Eat carbohydrates in abundance (yes ,No)	(92%, 4%)
Eat more fats (yes ,No)	(80%, 20%)
Playing sports (yes ,No)	(46%, 54%)
His inheritance in acne (yes ,No)	(48%, 52%)
Female adrenal hormones disorder (yes ,No)	(38%, 62%)
Stress (yes ,No)	(70%,30%)
Abundant consumption of milk and milk products (yes ,No)	(88%,12%)
Eat chocolate a lot (yes ,No)	(76%, 24%)
Exposure to chronic infections (yes ,No)	(62%, 38%)
The use of cosmetics a lot or of a poor quality (yes ,No)	(30%, 70%)
Polycystic ovaries for females (yes ,No)	(6%,94%)

3.2. Examination the distribution of data in the studied groups:

A box plot was used to visually show the distribution of data through displaying the data quartiles (or percentiles) and averages . Box plots show the five-number summary of a set of data: including the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score . The median is the average value from a set of data and is shown by the line that divides the box into two parts .

In statistics, dispersion (also called variability, scatter, or spread) is the extent to which a distribution is stretched or squeezed. The smallest value and largest value are found at the end of the 'whiskers' and are useful for providing a visual indicator regarding the spread of measurements . On the other hand, figures also indicated that the interquartile ranges of the boxes regarding patients have more dispersion of a data set then health control with indicated more variability .

All control group were demonstrated a symmetric distribution where most of the measurements . Cluster around the central median and the probabilities for values further away from the mean taper off equally in both directions. Extreme values in both tails of the distribution are similarly unlikely .

Figures (3.1 & 3.2), 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 .

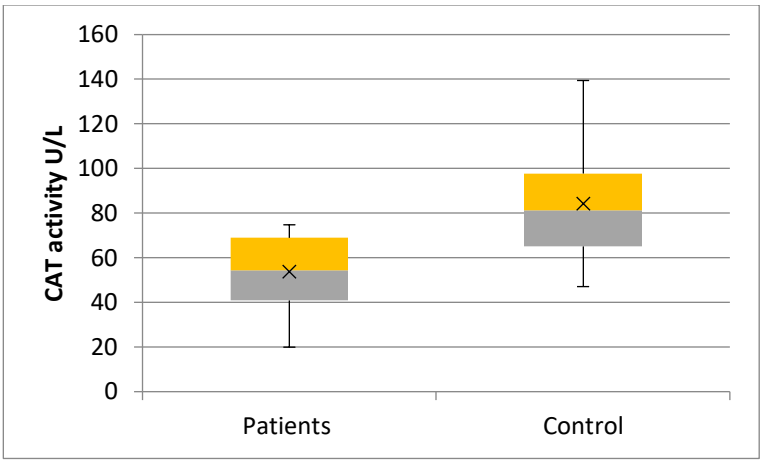


Figure (3.1): Distribution of serum activity of antioxidant enzymes catalase activity in acne patients group compared to the healthy group .

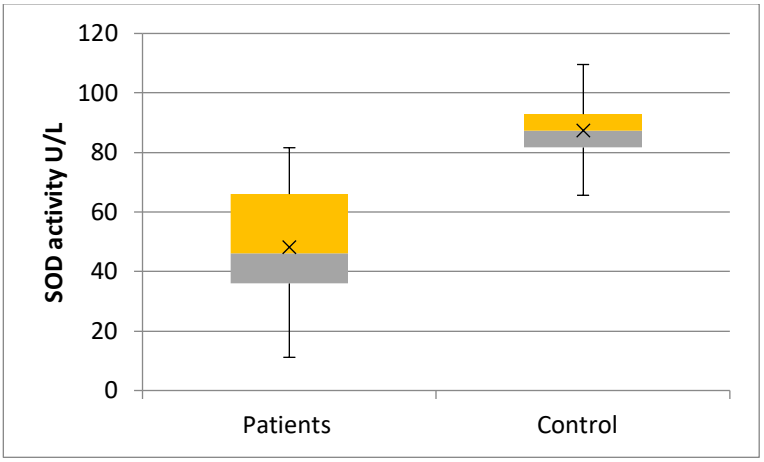


Figure (3.2): Distribution of serum activity of antioxidant enzymes superoxide dismutase activity in acne patients group compared to the healthy group.

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) as shown in figures (3.3 & 3.4) .

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 .

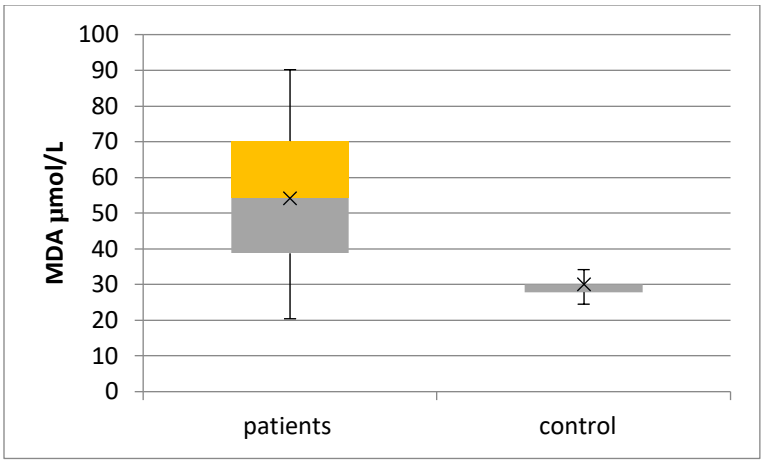


Figure (3.3): Distribution of serum activity of oxidative stress markers MDA in acne patients group compared to the healthy group.

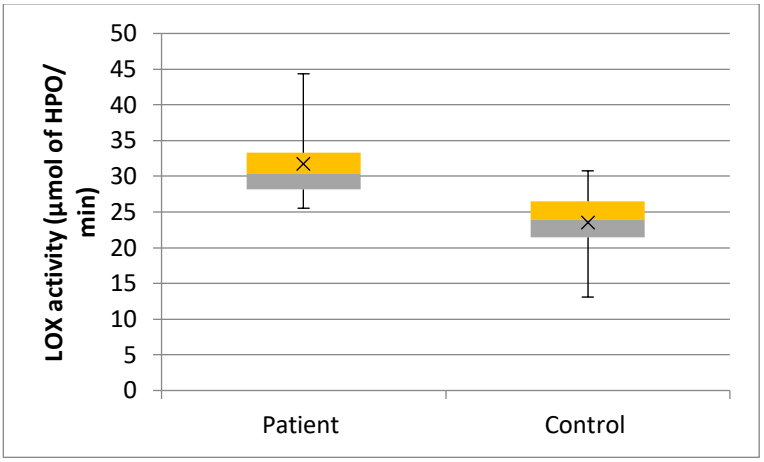


Figure (3.4): Distribution of serum activity of oxidative stress markers LOX activity in acne patients group compared to the healthy group.

Since the pathogenesis of this disease is multifactorial, many Compelling evidence suggests that oxidative stress is involved in the onset of acne **[74]**. 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 **[75] .**

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 **[76]** .

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 **[77]** .

The high plasma levels of MDA in our acne patients may be a result of cellular damage caused by ROS .

Many factores could be resulted in these reactive species, increasing level of ROS could be attributed to reduced levels of antioxidant enzymes **[55]** .

Toxic molecules and reactive oxygen species play a crucial part in the pathogenesis and severity of acne vulgaris and might be 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 **[78] .**

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 **[79] .**

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 **[51] .**

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, 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 **[80]** .

3.3. Examination the levels of oxidative stress markers and the activity of antioxidant enzymes based on severity of acne.

This study also examined the levels of oxidative stress markers and the activity of antioxidant enzymes in the major five types of acne lesion .

The levels of plasma oxidative markers in patients with acne of differing severities is shown in figure (3.5) .

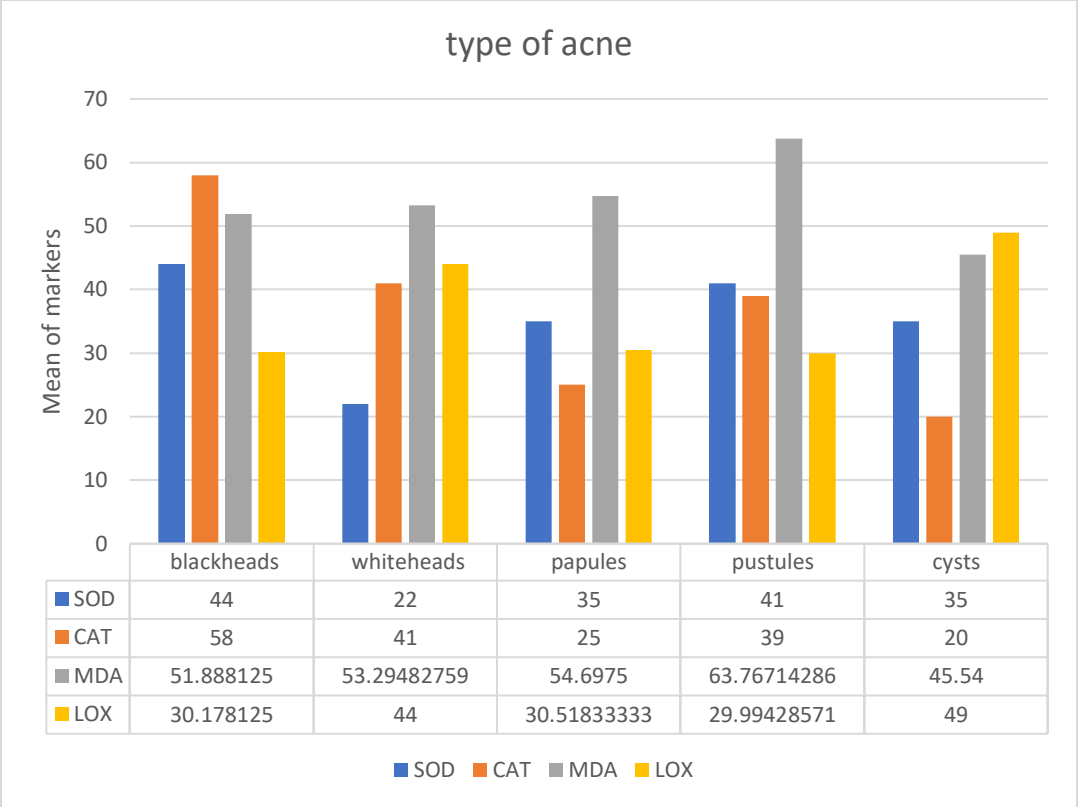


Figure (3.5): Levels of oxidative stress markers and the activity of antioxidant enzymes in the major five types of acne .

the levels of lipid peroxidation markers were found to be negatively correlated with the activities of CAT and SOD and correlated to the severity of disease **[81]** . Alterations in this normal balance,may occur due to elevated ROS production and/or decreased antioxidant levels, that can lead to a state of oxidative stress **[82]** .

The lowest level of sod was shown in whitehead acne type, while cysts type shown decrease level of CAT. Both former types were shown a higher level of MDA and LOX .

Previous studies have revealed that the level of lipid peroxidation increases in inflammatory diseases; moreover, its final product, MDA, is considered an indicator of the oxidative stress in the cells **[78]** .

Thus, the high plasma levels of MDA in acne patients may be a result of cellular damage caused by ROS .

These results suggest that acne is mediated, at least in part, by the increased generation of ROS, which may be attributed to reduced levels of antioxidant enzymes, including SOD or CAT. In various diseases, it has been observed that the SOD-CAT system may be affected in a way of increasing and decreasing or in two different directions **[55] .**

The alterations in oxidant/antioxidant status noted in this study may be utilized as a biomarker index for differentiating between varying levels of severity of acne and for evaluating the response to treatment .

This suggestion needs to be confirmed by conducting further studies on a larger number of acne patients. The alterations in the antioxidant enzyme activities in the plasma of acne patients might reflect a peripheral response of the organism to increase oxidative stress.However, when antioxidants levels are measured in plasma, it is not possible to determine the origin of these enzymes **[83] .**

It appears likely that these changes are not the cause but rather the consequence of cutaneous inflammations such as acne **[84] .** 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 .

3.4. 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 **[85]** . 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 **[86]** .

Figure (3.6): demonstrated a high level of lipid-peroxidizing enzyme (LOX) and final product of the process (MDA) in patients with acne who have high carbohydrate, fat and dairy product in their daily diet which associated with the alterations in this balance of oxidative and antioxidant .

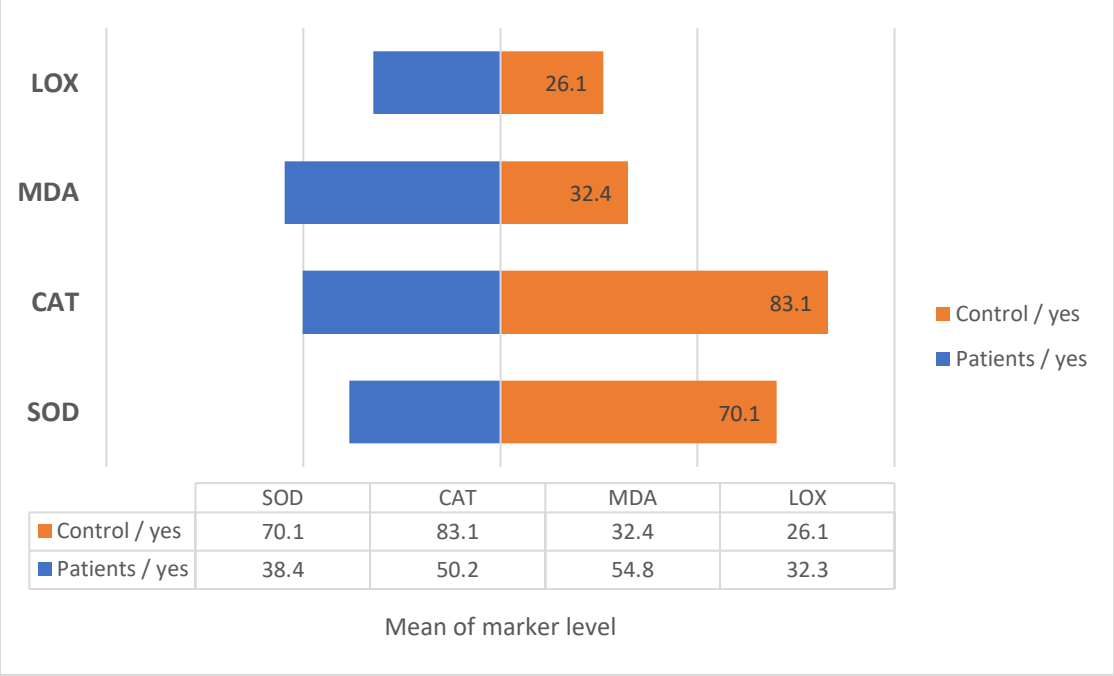
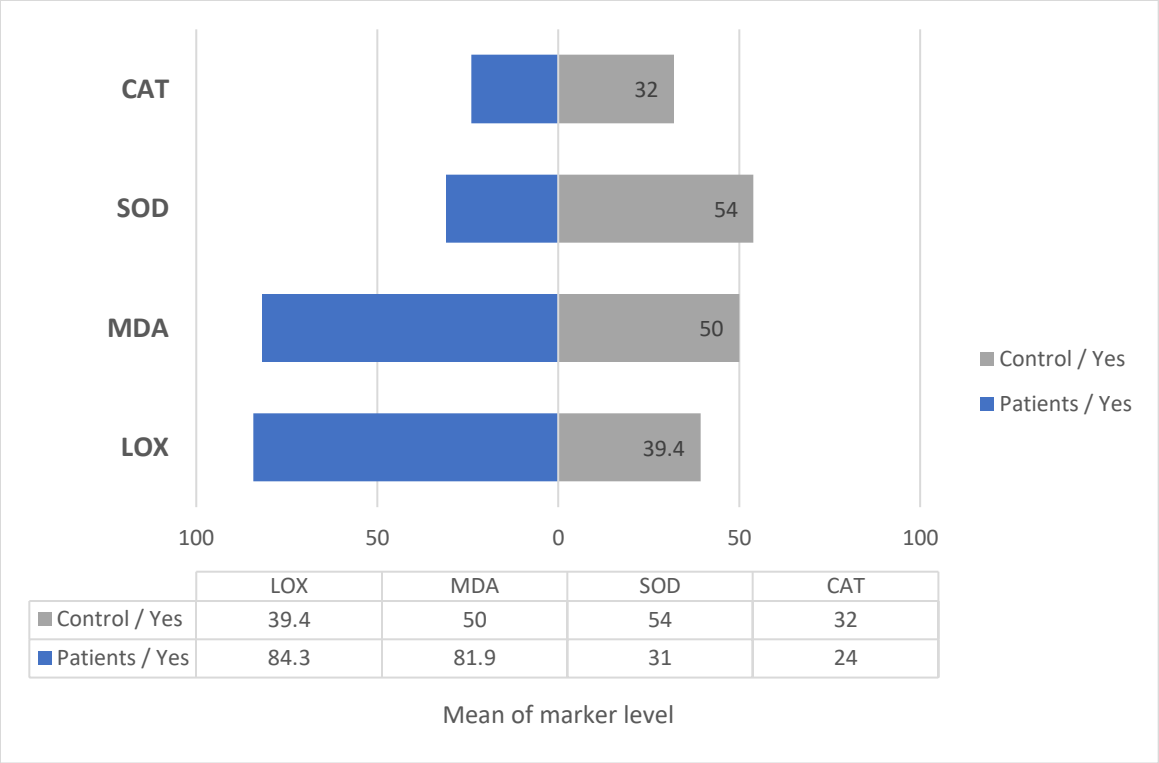


Figure (3.6): Effect of dietary factors on the oxidative stress/antixoidants levels in acne patients

In case of elevated glucose concentrations in the bloodstream, most cells might suffer from pronounced oxidative stress. Both direct damage by AGE(advanced glycation end-product) 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)(receptor of advanced glycation end-product), which results in the production of ROS **[87]** .

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 **[88] .**

On the other hand, the association between repeated inflammation and acne was also studied. Figure (3.7) indicats high levels of oxidative stress markers and low level of antioxidant activity in patient of acne compared to control group.



	LOX	MDA	SOD	CAT
Control / Yes	39.4	50	54	32
Patients / Yes	84.3	81.9	31	24

Figure (3.7): Effect of repeated inflammation on the oxidative stress/antixoidants 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 promotes inflammation by enhancing the levels of proinflammatory cytokines and the expression of cellular adhesion molecules and growth factors **[89] .**

3.5.Receiver operating characteristics of oxidative stress/antixoidants levels in acne patients

Receiver operating characteristics (ROC) curve analysis of oxidative stress makers and antioxidant were performed. The best area under the ROC curve (AUC) for the acne patients was for SOD ACTIVITY (AUC = 0.96, $p < 0.001$) Figure (3.8) .

ROC analysis indicated that SOD ACTIVITY <12 U/ml was predictive of increasing oxidative stress at the expense of antioxident with 98% sensitivity, 82% specificity as shown in table (3.3) .

Table (3.3): Differentiation power (area under the ROC peak, Sensitivity % and Specificity %) of the SOD ACTIVITY (U/ml) in acne patients

SOD ACTIVITY U/ml	AUP	Sensitivity %	Specificity %	Cut-off points	Asymptotic Sig	CI (95%)
	0.965	0.98	0.82	12	>0.001	0.929- 1.000

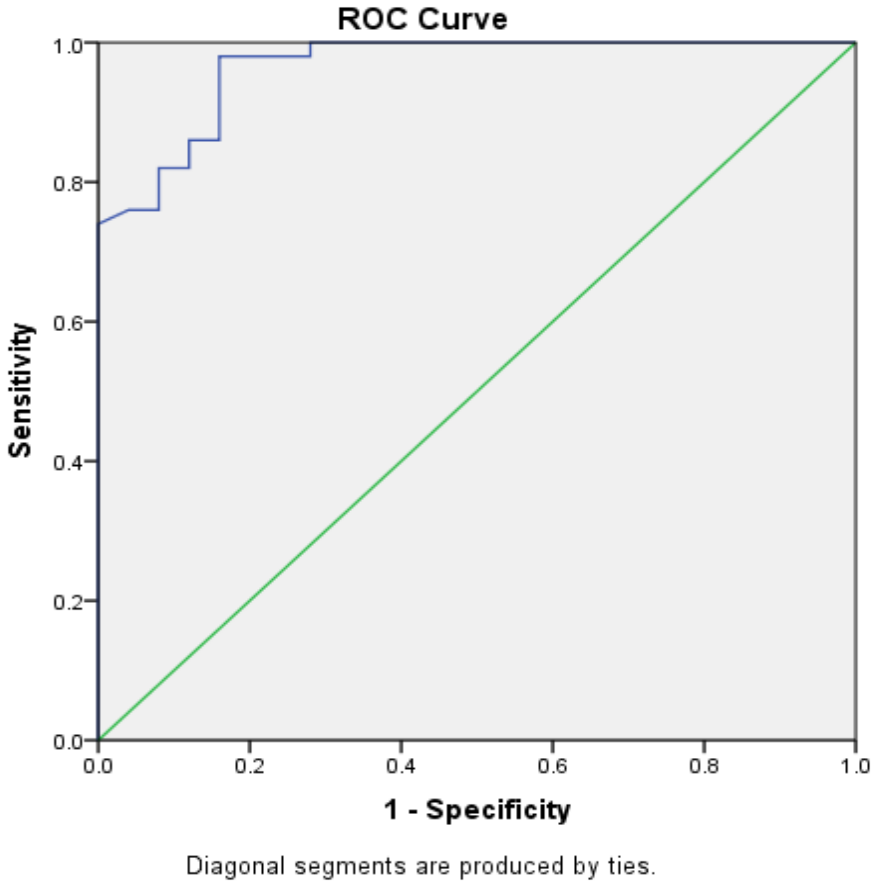


Figure (3.8): receiver operating characteristics (ROC) curve analysis of SOD

On the other hand, ROC analysis of the MDA demonstrated an area under the curve equal to (0.865) as shown in figure (3.9) when the MDA levels > 21.4(mol/L) in acne patients with 98% sensitivity, 76% specificity as indicated in table (3.4) .

Table (3.4): Differentiation power (Area under the ROC peak, Sensitivity % and Specificity %) of the MDA level (mol/l) in acne patients

MDA Con.(mol/L)	AUP	Sensitivity %	Specificity %	Cut-off points	Asymptotic Sig	CI (95%)
	0.865	0.98	0.76	21.4	>0.001	0.783- 0.946

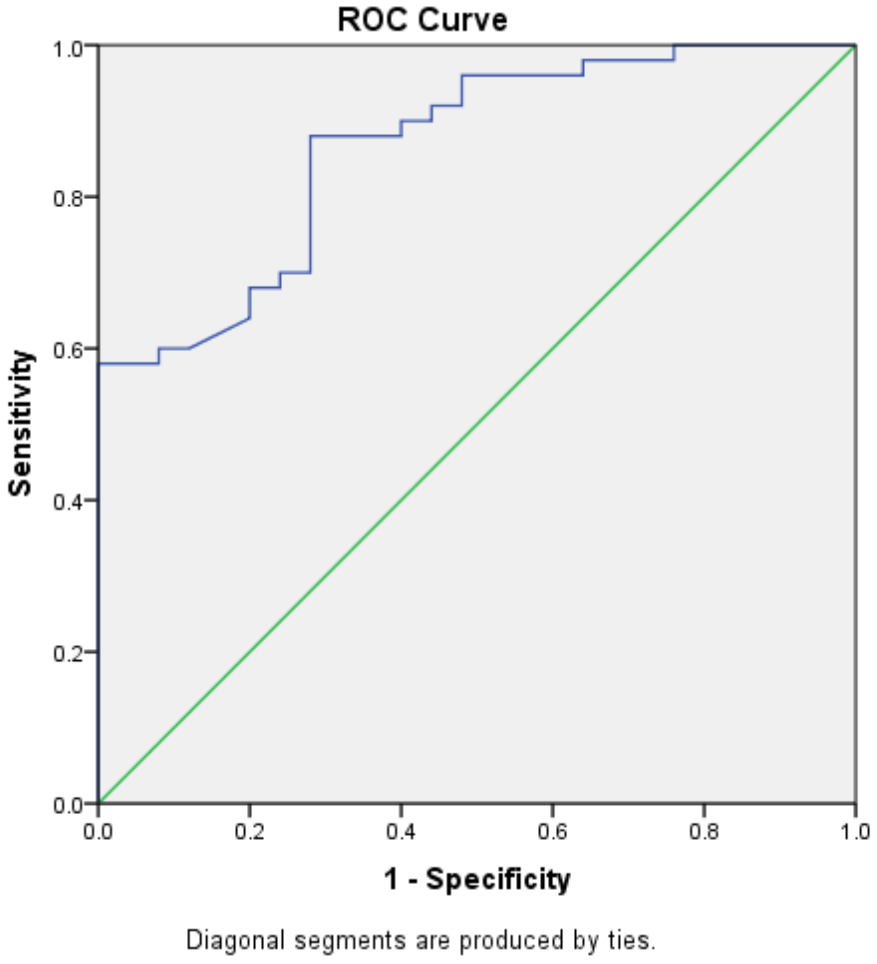


Figure (3.9): Receiver operating characteristics (ROC) curve analysis of MDA.

Further,figure (3.10) 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 (3.5)).

Table (3.5): Differentiation power (Area under the ROC peak, Sensitivity % and Specificity %) of the LOX activity (µmol of HPO/min) in acne patients.

LOX activity	AUP	Sensitivity %	Specificity %	Cut-off points	Asymptotic Sig	CI (95%)
	0.918	0.92	0.82	20	>0.001	0.853- 0.983

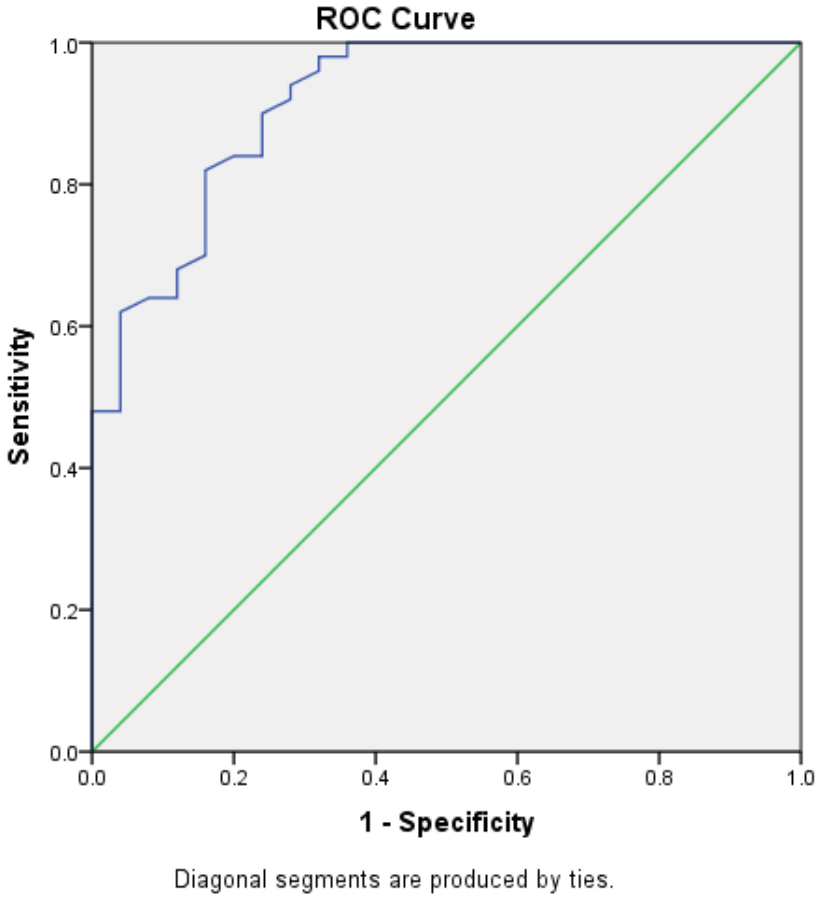


Figure (3.10): receiver operating characteristics (ROC) curve analysis LOX

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_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 **[51] .**

Therefore, the current study could suggest monitoring oxidative stress and antioxidant levels as a good biomarker for prognosis of acne.

Chapter Four

Conclusion
&
Future work

4.Conclusion and future work

4.1.Conclusion

- Results indicated that both serum SOD and CAT activities decreased 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.
- The levels of lipid peroxidation markers were found to be negatively correlated with the activities of CAT and SOD and correlated to the severity of disease. The alterations in oxidant/antioxidant status noted in this study may be utilized as a biomarker index for differentiating between varying levels of case severity.
- Furthermore, results corelated between the increasing level of lipid-peroxidizing enzyme (LOX) and final product of the process (MDA) with acne patients who have high carbohydrate, fat and dietary product in their daily diet.

4.2.Future works

- Emerging researches on the oxidative stress in acne with the brain-skin axis might be a potential avenues of approach the treatment of acne via specific nutrients, dietary modifications, oral and topical interventions.

- Studying the influence of individual antioxidant supplement in the reducing level of pro-oxidant activity and oxidative markers.

- Pre and post study for the acne severity assessment could be correlated with investigating daily dietary, weight loss or differences in protein and/or fiber content , amount of fat and carbohydrate.

Chapter Five

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5.References

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APPENDIX

Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbela

College of Medicine

Department of Chemistry and Biochemistry

Experimental data:

Study questionnaire

Sample data:

No.	Date

ID

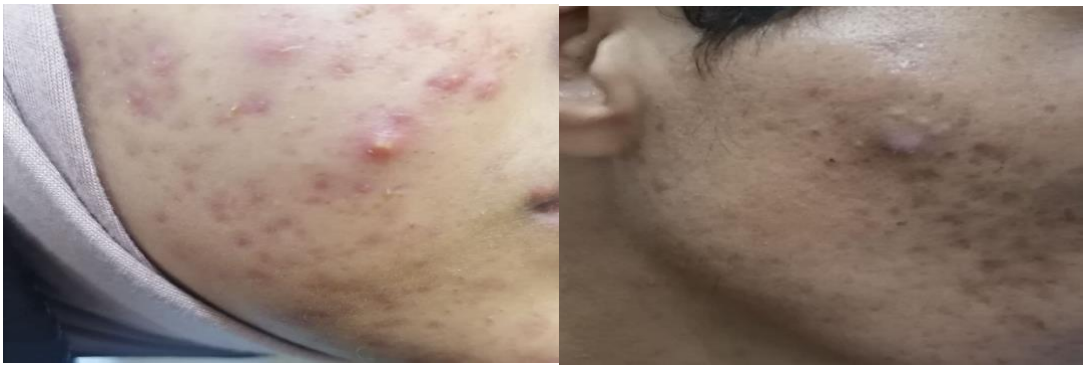
Patient name:	Gender:	Age :	Hight (m^2):	Weight(Kg):	Mobile:

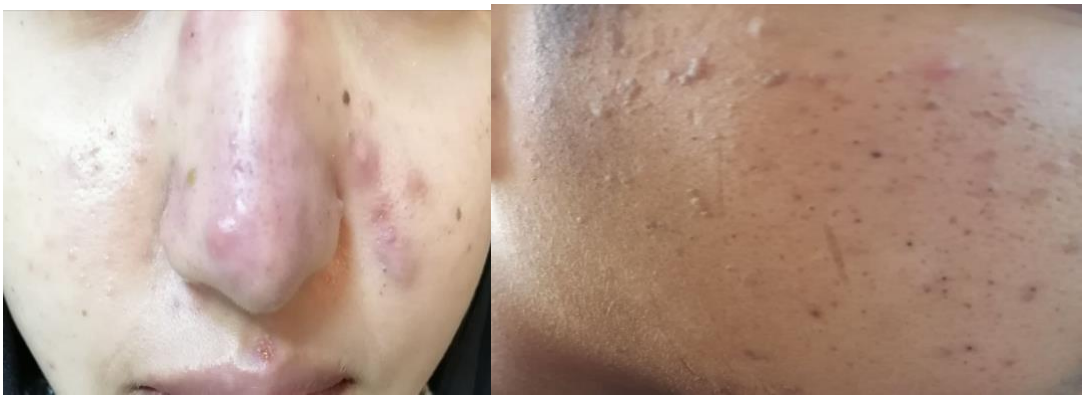
Adress:	Smoking:	Autoimmune disease:	Hypertensive:	Diabetes mellitus:

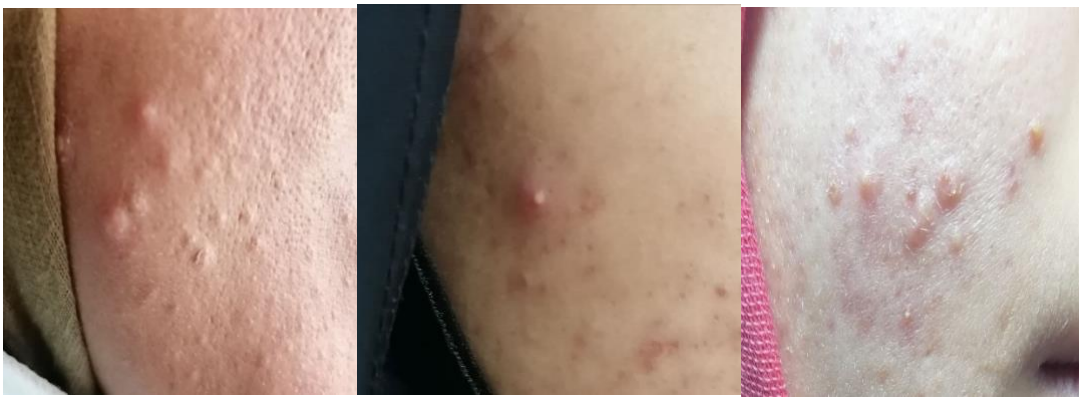
Disease state :

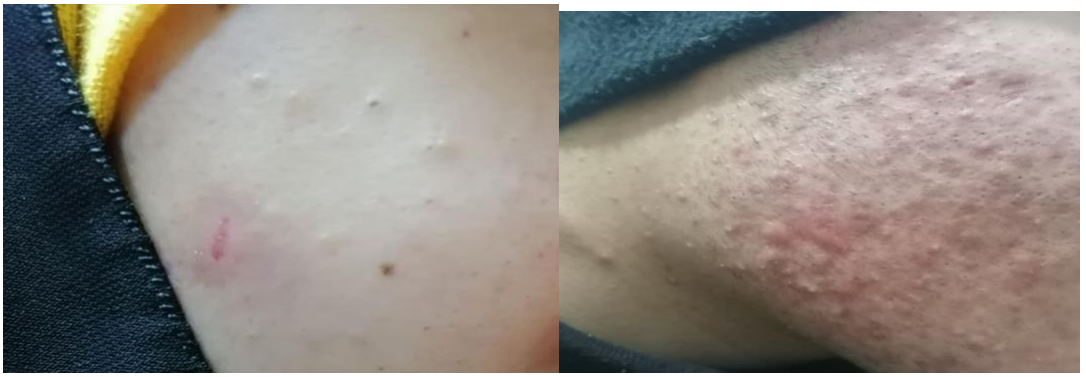
Whiteheads:	Blackheads:	Papules:
Pustules:	Nodules:	Cysts:

Questions	YES	No
Do you eat a lot of carbohydrates?		
Do you eat a lot of fat?		
Do you exercise regularly?		
Do you have hereditary acne?		
Do you suffer from a disorder of the adrenal hormones Do you suffer from stress?		
Do you consume a lot of milk and its products?		
Do you eat dessert a lot, do you suffer from chronic inflammation?		
Do you use too much or poor quality cosmetics?		
Do you suffer from PCOS?		









الخلاصة

حب الشباب هو التهاب مزمن للبصيلات الشعرية مع مسببات مرضية غير معروفة. تساهم أربعة عوامل رئيسية في التسبب في الإصابة بحب الشباب هي: فرط تنسج الغدد الدهنية , زيادة افراز الزهم، فرط التقرن في القناة الشعرية الدهنية ، الاصابه البكتيرية بواسطه **P. acnes** ، والالتهاب. ركزت الدراسات الحديثة حول المسببات المرضية لحب الشباب الشائع على دور الجذور الحرة للأكسجين والإنزيمات المضادة للأكسدة. عندما تصبح الإنزيمات المضادة للأكسدة غير قادرة على الضرر التأكسدي ، تبدأ الجذور الحرة للأكسجين في أكسدة الدهون في أغشية الخلايا والعضيات. يسبب الإجهاد التأكسدي أضرارًا لجميع المكونات الخلوية من خلال التأثيرات على الدهون والبروتينات والحمض النووي. من بين هذه التأثيرات ، تلف الدهون من خلال الإجهاد التأكسدي الناجم عن بيروكسيد الدهون الذي له صلة خاصة بحب الشباب. تم اقتراح التسبب الكيميائي لحب الشباب على أساس التحطيم التأكسدي للدهون في الجلد. قد تعمل بيروكسيدات الدهون ، وهي منتجات بيروكسيد الدهون ، كمسبب لحب الشباب أو كعوامل مسببة لحب الشباب أو كليهما. تأتي البيانات الداعمة لفرضية بيروكسيد الدهون من دراسة تظهر أن بيروكسيد الدهون يحدث في حب الشباب وتلف الجذور الحرة الخاصة بالموقع ومنتجات بيروكسيد الدهون قد تتضمن بدء الالتهاب. نظرًا لأن مسببات حب الشباب غير مفهومة تمامًا ، ولم يتم تحديد سبب رئيسي واحد ، لذلك ، هدفت هذه الرسالة إلى التحقيق في مستوى المادة المسببة للالتهابات التي قد تسبب حب الشباب ، وكذلك فحص نشاط مضادات الأكسدة ودورها في تنظيم الإجهاد التأكسدي لمرضى حب الشباب .

تضمنت هذه الدراسة جمع البيانات الطبية ل 75 عينة (50 مريض بحب الشباب ، 25 حالة سيطرة) من مدينة الامام الحسين الطبية في كربلاء –العراق **(**في الفتره من شهر كانون الثاني الى شهر اذار سنة ٢٠٢١**)**. تم تقسيم حالات حب الشباب إلى مجموعات فرعية على أساس شدة المرض. تم قياس مستوى المؤشرات الحيوية في المصل للمعلمة التالية : نشاط **SOD** و **CAT** و **LOX** في الدم ومستويات **MDA** ، والتي تم إجراؤها باستخدام تقنية مقياس الطيف الضوئي. تم تقييم العلاقة بين المؤشرات البيوكيميائية وشدة المرض وتم تقييم كفاءة قيمة التنبؤ باستخدام منحنى خاصية تشغيل المستقبل (**ROC**).قد يمثل **LOX** و **SOD** في مصل الدم أداة طبية مفيدة سريريًا توفر اشارات مهمة في المرضى الذين يعانون من حب الشباب. لذلك ، يمكن أن تقترح الدراسة الحالية مراقبة الإجهاد التأكسدي ومستويات مضادات الأكسدة كمؤشر بيولوجي جيد لتشخيص حب الشباب.

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دور العوامل الموطئه للألتهابات والأجهاد التأكسدي في ظهور حب الشباب

رسالة مقدمة

الى مجلس كلية الطب/ فرع الكيمياء و الكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل

مروه صاحب محمد مهدي

بكالوريوس علوم كيمياء – جامعة كربلاء – 2013

اشراف

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