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Assessment the role of survivin and IGF-1 with foxo1 in patient with Acne Vulgaris in Babylon City

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

صدق الله العلي العظيم

(المجادلة-11)

Supervisor Certification

We certify that this thesis entitled " **Assessment the role of survivin and IGF-1 with foxo1 in patient with Acne Vulgaris in Babylon City** was carried out under our supervision at the College of Medicine, University of Babylon, as partial fulfilment for the requirement of the degree of Master of Science in Clinical Biochemistry

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Dedication

To my brother (alshaheed) Mahdi

To my life Father & Mather

My dear wife and my eyes :Layan &Mahmoud

My sisters & brather Karar

To everyone I love.

Hamza 2022

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل\كلية الطب
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تقييم دور السورفايفين (survivin) وعامل النمو1 الشبيه بالانسولين (IGF1) وعامل النسخ (FOXO1) لدى مرضى حب الشباب في محافظة بابل

رسالة

مقدمة إلى عمادة كلية الطب في جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستير في العلوم
الكيمياء الحياتية السريرية

من قبل

حمزة محمود حمزة علوان

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Summary

Acne vulgaris (AV) is a cutaneous chronic inflammatory disorder with complex pathogenesis it is the eighth most common disease worldwide. Acne vulgaris occurs against the background of seborrhea a genetically determined disease characterized by increased secretion of chemically altered sebum by the sebaceous glands and the appearance of acne in areas of the skin that are richest in sebaceous glands, A patient with acne usually presents with a history of papule, pustules most commonly affecting the face, back, chest and shoulders. Systemic symptoms are often absent, but the patient may describe local symptoms of pain, erythema or tenderness. actors in acne vulgaris insulin growth facter -1 ,survivin and the Forkhead transcription factor.

The study's objective is to investigate the relationship between serum levels of insulin growth facter -1 (IGF1), survivin and forkhead transcription factor(foxo1) in patients with AV and healthy controls. This study was designed as a case-control study. 45 acne patients were involved in this study (20 females and 25males) in addition to 45 healthy controls (20 females and 25 males) well-matched with the patients in age and gender. Age was (15 – 30) years mean age 21.27 ± 3.19 years, body mass index(BMI) with (22.92 ± 3.12) Kg/m2.Samples have been taken Dermatology clinics at Al- Musayyib General Hospital, Marjan Teaching Hospital and Imam Al-Sadiq Hospital. The levels of IGF1,Survivin and foxo1 were measured by enzyme-linked immunosorbent assays(ELISA) technique, the levels insulin growth facter -1 ,survivin and the Forkhead transcription factor were significantly more than healthy people, as the value of $P \leq 0.05$. Relationship showed significant association between scale and category vairable in level of survivin between Gander ($p = 0.017$) but no significant association in level of IGF-1 and foxo1.The results show no significant association in level of IGF-1, survivin, and Foxo1 between body mass index(BMI) subgroup, the results show no significant difference in level of IGF-1, and foxo1 where p value is(>0.05) and significant in survivin of severity of acne, where p value is(<0.05).

There were no significant association between BMI and AV. there were positive correlation between IGF1 and Foxo1, where p value is (<0.05) and positive correlation between IGF1 and survivin ,and positive correlation between survivin and foxo1

the ROC curve test showed a good discriminative value of survivin and Foxo1 between AV patients and controls. Our findings suggest that IGF-I and survivin could play potential roles in the pathogenesis of acne vulgaris.

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List of Abbreviations

Abbreviation	Details
A	
ACTH	adrenocorticotropic hormone
ASC	adipose stromal cells
AV	Acne volgaris
AUC	area under curve
B	
BIRC5	baculovirus inhibitor of apoptosis repeat containing5
BMI	Body mass index
C	
CPC	chromosomal passenger complex
CRH	Corticotropin-releasing hormone
E	
ELISA	enzyme-linked immunosorbent assays
F	
FOXO1	The forkhead transcription factor
G	
GH	Growth hormone
GI	glycemic index
I	
IGF1	Insulin-like growth factor1
IGFBP1	insulin growth factor binding protein 1
IAP	Inhibitor apoptosis
M	
MAP	mitogen-activated protein
MTORC1	mammalian target of rapamycin complex 1

P	
PI3K	Phosphoinositide 3-kinase
PTEN	Phosphatase and tensin homolog
R	
RBP4	retinol binding protein 4
ROS	reactive oxygen species
S	
SD	standard deviation

Chapter One

Introduction and Literature Review

1. Introduction

1.1 Histology of Normal Skin

Skin is the largest human organ and its main role is to protect the organism from external factors and to prevent water and electrolyte loss. The uppermost layer of skin, the stratum corneum, represents the first line of the skin's barrier function [1].

The sebaceous gland is most commonly found in association with a hair follicle. Its traditional function is the holocrine production of sebum, a complex mixture of lipids, cell debris, and other rather poorly characterized substances[2]. the most important feature of the skin is its ability to self-remodel and regenerate, and to replace the tissue that is continuously being lost[3].

Human skin is composed of countless neural sensors that can perceive various stimuli, such as pressure, temperature, and texture of an object[4].

1.1.1Type of Skin Layer

1.1.2 The Epidermis

The epidermis is the most superficial and biologically active of these layers as the basal layer of the epithelium (stratum basale) is constantly renewing, The superficial epidermis undergoes a process of cornification which is one of the adaptive processes to providing the body with a barrier to the elements[5]. The epidermis is an important site of interactions between microbes and the host and keratinocytes are one of the skin components that participate in host defense [6].

1 .1 .3 The Dermis

The dermis is a connective tissue layer sandwiched between the epidermis and subcutaneous tissue. The dermis is a fibrous structure composed of collagen, elastic tissue, and other extracellular components that includes vasculature, nerve endings, hair follicles, and glands. The role of the dermis is to support and protect the skin and deeper layers, assist in thermoregulation, and aid in sensation[7].

1.1.4 The hypodermis

The hypodermis is the third layer of the skin located directly below the dermis and serves to connect the skin to the underlying fascia. Importantly, the hypodermis contains adipocytes, vasculature, nerves, and adipose stromal cells (ASC) ,notably, this layer is not only critical to maintain the mechanical and thermoregulatory properties of normal skin, [8]. As shown in the figure below

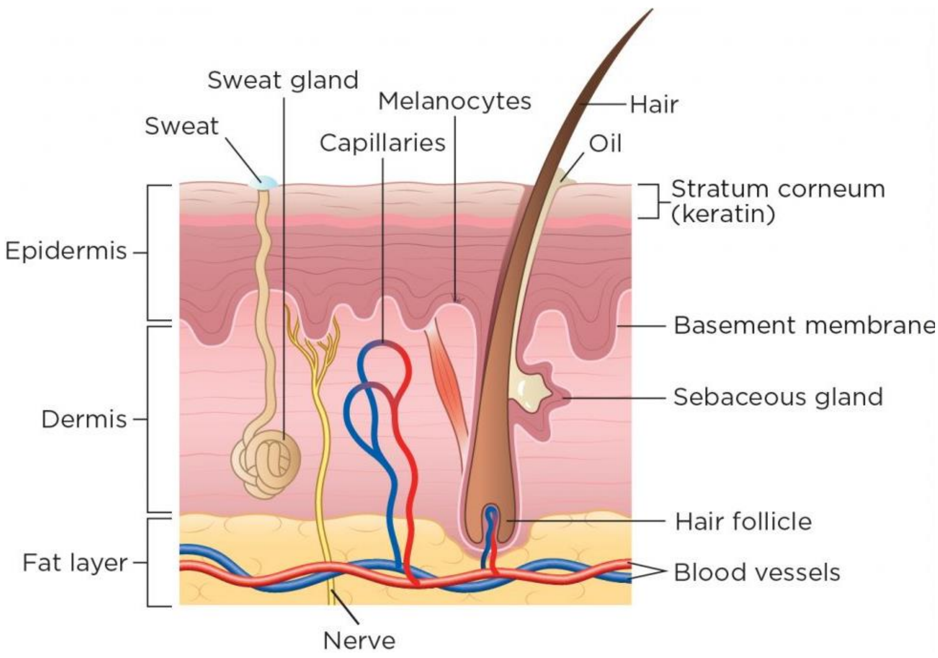


Figure1.1 The layers of skin[9].

The skin appendages include sweat glands, nails, and the pilosebaceous unit of the skin, comprised of the hair shaft, hair follicle, sebaceous gland, and arrector pili muscle these appendages derive from a down growth of the epidermis beginning in the third month of fetal life.The pilosebaceous unit is found in nearly all regions of the skin except for the lips, palmar and plantar surfaces, and is most dense on the scalp[10].

The epidermis is comprised of 4 or 5 layers, depending on the location of the skin sampled. These layers from deep to superficial are stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum[11].The facial sebaceous glands in Asian skin have been detected by three-dimensional ultrasound microscopy in a study, which observed cauliflower-shaped sebaceous glands in men while more cylindrical and smaller sebaceous glands in women than the young men[12].

1.2 Acne Vulgaris

Acne vulgaris (AV) is a cutaneous chronic inflammatory disorder with complex pathogenesis it is the eighth most common disease worldwide, disease burden of acne such as anxiety, reduced self-esteem, and facial scarring lowers the life quality of acne patients[13-14].

occurs most prominently at skin sites with a high density of sebaceous glands such as the face, back, and chest[15].

Acne occurs due to increased sebum production, hypercornification of the pilosebaceous duct, the activity of *Staphylococcus epidermidis* and *Propionibacterium* acnes bacteria and the inflammation of the pilosebaceous unit [16].

Despite being a frequent and nonthreatening life condition, acne has a significant psychological impact and comorbidity [17].

The negative psychological effect of acne vulgaris can be profound and long-lasting, although acne is not an infectious disease, oral antibiotics have remained a mainstay of treatment over the last 40 years [18-19]. Acne vulgaris is triggered by *propionibacterium* acnes in adolescence,under the influence of normal circulating dehydroepiandrosterone [20].

Hyperseborrhea has been considered as a major etiopathogenetic factor of acne. However, changes in sebaceous gland activity not only correlate with seborrhea but also with alterations in sebum fatty acid composition. The oxidant/antioxidant ratio of the skin surface lipids and alterations of lipid composition are the main players in the induction of acne inflammation [21].

Some of the key mechanisms involved in the development of acne include disturbed sebaceous gland activity associated with hyperseborrhoea (that is, increased sebum production) and alterations in sebum fatty acid composition [22].

Clinical features are whiteheads (closed plugged pores),blackheads (open plugged pores),papules, pustules ,nodules and (cystic lesions) [23].

Acne vulgaris occurs in 79-95% of adolescents and young adults. Often, however, it develops in infants and children before adolescence. under certain circumstances, especially when the symptoms of premature puberty or virilization coexist [24].

It is proposed that the sebaceous glands that cause acne are present on the face and forehead as they confer a selective advantage by 'lubricating' the widest parts of the new born baby to ease the passage of childbirth. Later in life, sebaceous glands may be inappropriately and pathologically primed, driven by a combination of hormones, diet and lifestyle to create acne [25].

As increased value is put on appearance, adolescents with acne may have a low quality of life with regard to their emotional state. Therefore, there is a high demand for an efficacious treatment for acne patients [26].

People with acne vulgaris report a lower level of satisfaction with life and are more frequently classified as having Type D personalities than those without acne [27].

1.2.1 Etiology of Acne Vulgaris

As the pathophysiology of acne is complex and multifactorial, the continued influx of new basic science and clinical information requires careful analysis before drawing conclusions about what truly contributes to the development and progression of this chronic disease [28].

Acne vulgaris occurs against the background of seborrhea a genetically determined disease characterized by increased secretion of chemically altered sebum by the sebaceous glands and the appearance of acne in areas of the skin that are richest in sebaceous glands,The sebaceous glands are abundantly supplied with blood from the superficial and deep dermal plexuses. They are involved in the excretion of various metabolic products, as well as toxic and medicinal substances [29].

Staphylococcus epidermidis and *Cutibacterium acnes* (C. acnes; formerly *Propionibacterium acnes*) are two major inhabitants of the skin that are thought to contribute to the disease but are also known to promote health by inhibiting the growth and invasion of pathogens Because *C. acnes* is ubiquitous in sebaceous-rich skin, it is typically labeled as the etiological agent of acne yet it fails to fulfill all of Koch's postulates [30].

One of the most disputed points, however, is the role of the gram-positive anaerobic bacterium *Propionibacterium* acnes in the development of acne, particularly when this organism is also found in normal sebaceous follicles of healthy skin[31]. During puberty, alteration of the sebaceous lipid profile, called dysseborrhoea, stress, irritation, cosmetics and potential dietary factors lead to inflammation and formation of different types of acne lesions[32]. Oxidative stress and inflammation set the stage for all subsequent pathogenic factors leading to acne , Oxidative stress is an imbalance between oxidants (free radicals)and antioxidants in favor of free radicals, Cellular injuries or Diseases, 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 [33].

Diet is frequently incriminated as a cause of acne vulgaris. In particular, chocolate and greasy food are often mentioned as the main culprits,In an early study published in 1983, 32% of patients believed that dietary factors would precipitate acne formation and 68% specifically stated chocolate as a causal factor [34].

1.2.2 Epidemalogy and prevalenece of Acne Vulgaris

Acne is estimated to affect 9.4% of the global population, making it the eighth most prevalent disease worldwide. Epidemiological studies have demonstrated that acne is most common in post pubescent teens, with boys most frequently affected, particularly with more severe forms of the disease. Recent general and institutional studies from around the world have shown that the prevalence of acne is broadly consistent globally (with the exception of specific population) [35].

Many papers have demonstrated that acne presentation is influenced by demographic factors. The onset of acne typically correlates with the onset of puberty, when sebum production increases as such, the prevalence of acne increases with increasing age [36].

A Kuwaiti study assessing over 3,700 patients with skin disorders indicated that approximately 75% of the study cohort had non-infectious disorders compared to 25% with infection-related skin ailments; the researchers found that acne was the second most common skin disorder (9.41%) after atopic dermatitis (11.07%) [37].

A meta-analysis can help inform the debate about the epidemiological evidence on dairy intake and development of acne[38].

Skin diseases have had a negative impact on human beings, both in acceptance of their own image and in quality of life. According to epidemiological studies, AV is a quite common condition, affecting approximately 80% of adolescents between 12 and 18 years of age. It is the most frequent cutaneous disorder seen by American dermatologists [39].

Acne is the most common diagnosis seen in United States dermatology ambulatory practices. It is uncertain whether there is truly an increase in prevalence or whether increased awareness is driving patients into the office to seek care [40]

Although prior studies have evaluated epidemiologic patterns of acne vulgaris in various ethnicities and regions, adequate understanding of the worldwide burden of the disease associated with patients in their late adolescence (17–19-year olds) remains lacking [41].

1.2.3 Classification of Acne Vulgaris

A usable and practical classification or grading system of diseases is important both in medical practice and research. It allows medical practitioners to treat diseases in an appropriate and effective manner. A generally accepted grading system makes the comparison between different studies on the same disease much easier. Multiple grading systems for acne have been proposed by several groups of dermatologists since the 1930s. Among those grading systems, 2 main methods of assessment were used: 1. global severity grading and 2. lesion counting. The former is based on the overall appearance that is divided into different levels by comparison with the descriptive text or standardized photography. The latter classifies based on the number of each acne lesion and then multiplying the number of each type of lesion by a given severity index [42].

Acne vulgaris is classified based on lesion morphology (comedonal, inflammatory, mixed, nodulocystic), distribution (location on face, trunk, or both), and severity (extent, presence or absence of scarring, postinflammatory erythema, hyperpigmentation). Although most acne does not require specific medical evaluation, medical workup is sometimes warranted [43].

Acne lesions typically occur on the face, chest, or upper back. The lesions may be noninflammatory closed comedones (i.e., papules formed by the accumulation of sebum/keratin within the hair follicle; also called whiteheads); open comedones (i.e., distension of the hair follicle with keratin leads to opening of the follicle, oxidation of lipids, also called blackheads); or inflammatory papules, pustules, nodules and cysts. Inflammatory lesions result from follicle rupture triggering an inflammatory response [44].

1.2.4 Pathogenesis Acne Vulgaris

Acne vulgaris (AV) is the most common skin disorder. It was traditionally thought that AV lesions developed after abnormal desquamation of the keratinocytes that line the sebaceous follicle, leading to hyperkeratinization and microcomedone formation. However, in recent years there has been a paradigm shift with regard to understanding the pathogenesis of AV, and it is now viewed as a primary inflammatory skin disorder. Research has implicated the presence of subclinical inflammation in the normal skin of acne patients, even before microcomedone formation[45].

The pathogenesis of acne vulgaris is multifactorial, Cytokines play an important role in the pathogenesis of acne vulgaris, together with other genetic and environmental factors[46].

Acne is one of the most frequent dermatological afflictions especially for people in their first 30 years of age. Several studies have shown that nutrition is one of the key factors involved in acne pathogenesis[47].

The relationship between diet and acne is highly controversial. Several studies during the last decade have led dermatologists to reflect on a potential link between diet and acne[48].

The role of dermocosmetics in dermatology, and in particular acne, is becoming more important as more research elucidates the mechanisms of action of products in the pathogenesis of acne. Therefore, it has become important for dermatologists to understand dermocosmetics to effectively and appropriately advise patients on their use [49].

Pathogenesis is complex and remains incompletely understood Follicular hyperkeratinization, sebum production, *Propionibacterium* acnes and inflammation appear to be involved, The role of factors such as vitamin D, diet, obesity and autoinflammation is evolving [50].

1.2.4.1 Hormones Implicated in Acne Pathogenesis

Hormones implicated in acne pathogenesis include androgens, estrogens, progesterone, insulin and insulin-like growth factor-1, Corticotropin-releasing hormone **(**CRH), adrenocorticotropic hormone (ACTH), melanocortins, glucocorticoids, and growth hormone (GH)[51].

Numerous triggers have been implicated in adult female acne including endogenous hormonal dysfunction and genetic predisposition [52].

1.2.5 Complications of Acne Vulgaris

Acne vulgaris is a common, often socially distressing skin condition primarily seen in young adults. Quality of life studies have shown that people with acne are more introverted with increased social setting anxiety compared to a control group. Unfortunately, patients with acne may have residual postinflammatory hyperpigmentation, amplifying impaired psychosocial effects[53].

Nearly 1% of people suffer acne scars, Reported prevalence rates of acne vary from 35% to more than 90% in adolescents, Scarring can result from inflammatory damage to connective tissue of the skin affected by acne. Eighty to ninety percent of people with acne scars have associated loss of collagen (atrophic scars), whereas a minority exhibit hypertrophic scars and keloids,Severe scarring caused by acne is associated with physical and psychological distress particularly in young adults, and often results in decreased self-esteem and diminished quality of life [54].

Atrophic scarring is often an unfortunate and permanent complication of acne vulgaris. It has high prevalence, significant impact on quality of life, and therapeutic challenge for dermatologists. The treatment of atrophic acne scars varies depending on the types of acne scars and the limitations of the treatment modalities in their ability to improve scars [55].

Most people who experience the acne suffer from damage under the surface of their skin which causes saucer-like depressions or pits on their skin. Sometimes the skin loses its underlying support and develops fibrous bands of tissue between the skin and subcutaneous layer, which pull on the epidermis and cause a wavy texture called as rolling scar. Treatment of acne scars is a therapeutic challenge that may require multiple modalities [56].

1.2.6 Clinical Diagnosis of Acne Vulgaris

Acne may be detected in more than 85% of adolescents, and approximately 50% of the cases face this problem until adulthood, , Acne frequently affects face, the presenting part of the body and may cause shame, a sense of guilt and social isolation. It is usually observed during adolescence, which is an important period for the development of self-identity and external appearance, with prominent social and physical changes in children's lives [57].

A patient with acne Systemic symptoms are often absent, but the patient may describe local symptoms of pain, erythema or tenderness. Additionally, acne can have a psychological impact, regardless of the severity of disease. The patients with acne were further subdivided into the following subsets using the International Consensus Conference on Acne classification system: “mild” (few to several comedones, papules, and pustules; no nodules); “moderate” (several comedones, papules, and pustules; few to several nodules); and “severe” (numerous comedones, papules and pustules; many nodules) [58].

Comedones are the most basic acne lesion and can be open or closed. Closed comedones (whiteheads) are small plugged follicles whose contents are not exposed to the skin surface. Open comedones (blackheads) are small follicles with dilated openings onto the skin.

Papules are small, usually red, raised elevations of the skin.

Pustules resemble papules but have a central pocket of pus.

Nodules and cysts are larger swellings usually more than 5 mm in size [59].

Comedonal acne is more typical in young adolescents, but can occur in combination with inflammatory papules and pustules at any time[60].

1.2.7 Treatment of Acne Vulgaris

Treatment methods depend on the clinical features and variants of acne Topical or/and systematic treatments are used to treat acne. The response of patients to treatment is considerably different. Usually more than one treatment modality is employed to treat acne and best results are achieved when treatments are individualized on the basis of clinical evaluations. Retinoids are the mainstay of therapy in patients who only have comedones. They are capable of reducing inflammatory lesions and the number of comedones (40% - 70%)[61-62]. Although antibiotic is commonly used to treat acne, limited transport of drug to the lesions within the pilosebaceous unit leads to poor bactericidal effect[63].

Acne vulgaris is a chronic disease that requires prolonged therapy for a satisfactory outcome. Treatment adherence in patients is a major problem, particularly for topical treatments, owing to side effects and the prolonged treatment time. Insufficient adherence leads to recurrence of acne, patient dissatisfaction, and increased medical costs. Numerous studies have reported low adherence rates for acne treatments, with the United States having the lowest rate of 11.74% [64].

It has also been shown that a low glycemic index (GI) diet induced an improvement in acne severity and a parallel improvement in insulin sensitivity in young males with acne vulgaris[65].

Although acne was earlier considered to be merely a cosmetic affliction, the psychosocial effects of the disease have now been scientifically proven. Studies have shown these effects to improve when acne is treated, thus, it is imperative that quality of life (QoL) issues of acne are taken into consideration for a wholesome management of the patients.[66]

Modern acne treatments are based also on the use of moisturizers, cleansers, In choosing the right cleanser, it is important to consider some aspects: the interaction between skin type and the cleanser, the optimal time and method of cleaning and the cosmetic perception of the patient [67].

Most acne patients remit spontaneously, for the ones that do not or are unresponsive to conventional therapy or have obvious cutaneous signs of hyperandrogenism, hormonal therapy is the next option in the therapeutic ladder. It is not strictly indicated for only those patients who have cutaneous or biochemical evidence of hyperandrogenism, but can be used even without any evidence of hyperandrogenism, for therapy-resistant acne. It can be prescribed as monotherapy, but when used in combination with other conventional therapies, it may prove to be more beneficial. Hormonal evaluation is a prerequisite for hormonal therapy, to identify the cause behind hyperandrogenism, which may be ovarian or adrenal[68]. Successful acne outcomes are influenced by compliance with topical regimens that can commonly cause skin barrier disruption, leading to dryness and irritation. Consequently, calming inflammation as well as maintaining skin hydration and barrier repair are of primary importance when treating acne. Probiotics modify several factors in the pathophysiology of acne development and can potentially improve compliance as well, the increasing resistance to antibiotics has decreased their effectiveness in treating acne. as viable microbial dietary supplements, probiotics provide health benefits through fighting pathogens and maintaining the homeostasis of the gut and skin microbiome [69-70].

1.3 Survivin

Survivin (also known as BIRC5) is an evolutionarily conserved eukaryotic protein that is essential for cell division and can inhibit cell death. Normally it is only expressed in actively proliferating cells, but is upregulated in most. When survivin (BIRC5) was first described, its discovery sparked considerable interest from oncologists and cell biologists, an interest that persists today. survivin is a small protein [142 amino acids (aa); 16.5 kDa] with multifunctional domains. Its N-terminal two-thirds comprise a globular baculovirus inhibitor of apoptosis repeat (BIR) domain (aa 20–90) [71].

Regulation of cell division is recognized as prominent function of Survivin. Normal cells show cell cycle dependent synthesis, expression and degradation of survivin. survivin forms an integral component of chromosomal passenger complex (CPC) which ensures proper segregation of chromosomes and cytokinesis during cell division. Overexpression of survivin inhibits both intrinsic and extrinsic pathways of apoptosis, depletion of survivin in human cells induces defects in apoptosis and multiple defects in cell division[72].

It is highly expressed in most human neoplasms, but its expression is very low or undetectable in terminally differentiated normal tissues. Survivin has been shown to inhibit cancer cell apoptosis and promote cell proliferation. The overexpression of survivin closely correlates with tumor progression and drug resistance. Because of its key role in tumor formation and maintenance, survivin is considered as an ideal target for anticancer treatment. However, the development of small-molecule survivin inhibitors has been challenging due to the requirement to disrupt the protein-protein interactions. Currently only a limited number of survivin inhibitors have been developed in recent years[73].

There are several survivin inhibitors under clinical evaluation, although more specific and efficient survivin inhibitors are being developed. Moreover, novel combination regimens targeting survivin together with other therapeutic approaches are currently being designed and assessed. recent progress in the therapeutic options targeting survivin for cancer treatment is analyzed. Direct survivin inhibitors and their current development status are explored. Besides, the major signaling pathways implicated in survivin regulation are described and different therapeutic approaches involving survivin indirect inhibition are evaluated [74].

1.3.1 Overview of Survivin Structure and Functions

The gene encoding human survivin was cloned by Ambrosini *et al* in 1997. Survivin spans 14.7 kb at the telomeric end of chromosome 17 and encodes wild-type survivin protein of 142 amino acids in length9.As the smallest member of the IAP family, all survivin isoforms characterized so far contain only one of the characteristic N-terminal BIR (Baculovirus IAP Repeats) domains; and an alpha-helix replaces the IAP characteristic RING finger domain involved in zinc ion-binding with the BIR domain [75]. As shown in the figure below

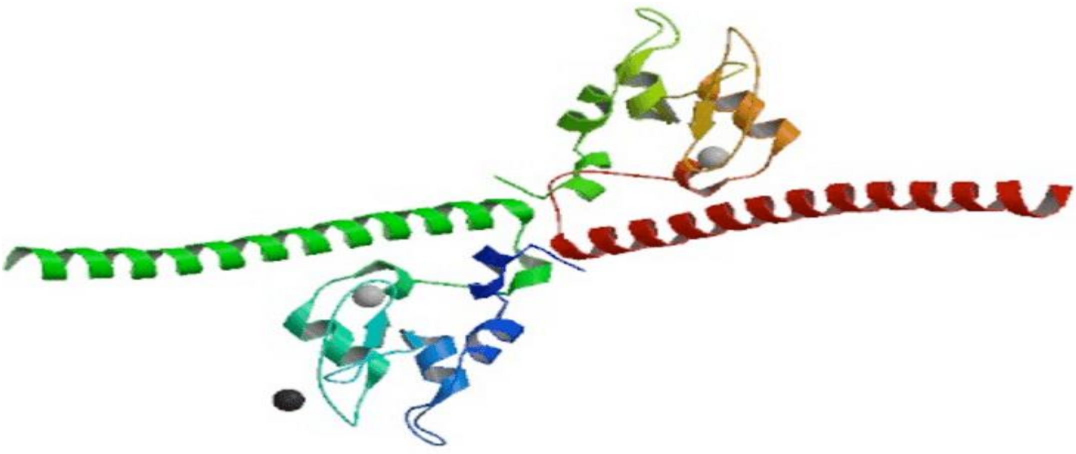


Figure 1-2 structure of Survivin [76]**.**

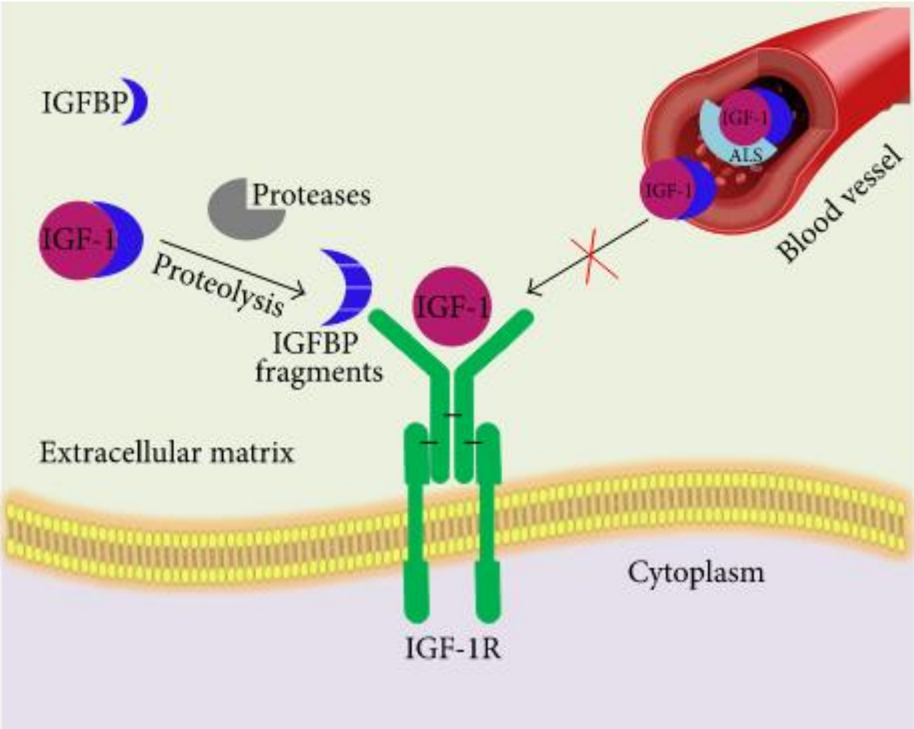
1.3.2 Role the Survivin in Acne Vulgaris

Survivin has been found to be increased in keratinocyte proliferative and inflammatory states, which are deeply involved in the pathogenesis of the acne lesions. This may affect both the sebaceous gland (sebocyte survival) and the perifollicular dermal tissue (scar formation). Moreover, nuclear survivin expression has been observed in sebaceous hyperplasia and neoplasia [77].

During terminal differentiation, sebocytes accumulate abundant lipids and exhibit degeneration of their nuclei eventually leading to programmed cell death followed by holocrine secretion of sebum (s1). Epidermal keratinocytes undergo a unique form of terminal differentiation including programmed cell death, called cornification. Cyclic apoptosis is the principle of the hair cycle (s2). The balance of pro-survival signalling and programmed cell death secures sebocyte homoeostasis, which is disturbed in acne vulgaris [78].

1.4 Insulin-Like Growth Factor (IGF)-1

Insulin-like growth factor 1 (IGF1) is a polypeptide hormone structurally similar to insulin. It is central to the somatotropic axis, acting downstream of growth hormone(GH),It activates both the mitogen-activated protein (MAP) kinase and PI3K signaling pathways acting in almost every tissue in the body to promote tissue growth and maturation through upregulation of anabolic processes [79]. As shown in the figure below



1-3.Figure .The IGF axis: circulating IGFs are protected from degradation by forming complex with IGFBPs. IGFs, apart from their local functioning in an autocrine or a paracrine manner, enter the bloodstream, where they exist as binary complexes with each IGFBP. In addition, ternary complex also exists when the binary complexes with IGFBP-3 or IGFBP-5 interact with the acid labile subunit (ALS). After dissociation of ternary complex, the binary complexes of IGFBP-IGF are removed from the circulation and cross the endothelium to reach the target tissues and to interact with cell surface receptors[80].

Insulin-like growth factor-1 (IGF-1) belongs to the growth factor family, structurally resembling pro-insulin, enabling it to bind to insulin receptors. It plays an important role in many tissues including skin, particularly acne pathogenesis by stimulating sebum production and affecting androgen levels[81].

1.4.1 Metabolic of Insulin Like Growth Facter- 1

IGF-1 is responsible for stimulating growth of all cell types and causing significant metabolic effects.One important metabolic effect of IGF-1 is its ability to signal cells that sufficient nutrients are available for cells to undergo hypertrophy and cell division.These signals also enable IGF-1 to inhibit cell apoptosis and increase the production of cellular proteins, IGF-1 receptors are ubiquitous, which allows for metabolic changes caused by IGF-1 to occur in all cell types,IGF-1's metabolic effects are far-reaching and can coordinate protein, carbohydrate, and fat metabolism in a variety of different cell types, The regulation of IGF-1's metabolic effects on target tissues is also coordinated with other hormones such as growth hormone and insulin[82].

1.4.1 Relationship between Serum IGF-1 and Acne

There is increasing evidence in support of the interplay of insulin-like growth factor-1 (IGF-1) signaling during puberty, which may have a causal role in pathogenesis of acne by influencing adrenal and gonadal androgen metabolism[83].

Diet is considered to be one of the main factors influencing the induction and aggravation of acne, though this is still debatable. Recent research interests have focused on glycemic load, and hyperinsulinemia resulting from a high glycemic load has been linked to an increase in the concentration of insulin-like growth factor (IGF1, which has been reported to affect androgen metabolism and lipogenesis[84].

1.4.2 Mechanism Of insulin/IGF-1 leads to Acne Vulgaris

Abnormal hormones function hyperandrogenemia, hyperinsulinemia and an elevated level of insulin growth factor-1 (IGF-1) have the crucial role in the acne development as well. Hyperinsulinemia is responsible for increased proliferation and dysfunction of keratinocytes by stimulation of IGF-1 receptors.

Hypersecretion of IGF-1 leads to abnormal sebum production, hyperproliferation of sebocytes and lipogenesis, The synthesis of androgens is promoted by insulin and IGF-1. Moreover, IGF-1 enhances androgen receptor signal transduction. As a consequence, the elevated androgen level encourages hyperseborrhea. Acne vulgaris tends to be regarded as a disease of Western civilization due to the fact that the increased serum level of insulin/IGF-1 is in addition significantly correlated with a high intake of carbohydrates, insulinotropic milk and dairy products [61].

1.5 The Forkhead Transcription Factor (FoxO1)

The forkhead transcription factor (FoxOs) are a subclass of the larger family of forkhead. Mammalians express four members foxo1, foxo2, foxo3, and foxo6. The interest in foxo function stems mostly from their observed role in determining lifespan, where in model organisms, increased foxos activity results in extended lifespan[85]. foxo are important in many processes, such as reactive oxygen species (ROS) suppression, promotion of survival and longevity, engagement of autophagy and regulation of metabolism. The function of foxos is typically governed through post-translational modifications of the proteins, which, in turn, regulate foxos subcellular localization and/or transcription Different subtypes of foxos proteins have different functions in different diseases. FoxO1 is considered to be a representative member of the foxo family, and has key transcription regulatory activities foxos are homeostatic factors in healthy skin and in skin disorders, An IGF1/foxo1/p63 signaling axis regulates epidermal morphogenesis[86].

The ubiquitously expressed forkhead box, class O (foxo) transcription factors act as signaling integrators in extensive transcriptional networks, ensuring maintenance of cell and tissue homeostasis over time and in response to environmental challenges. Proteins whose biosynthesis is controlled through foxos fulfil key functions in antioxidant defense, metabolism, cell cycle regulation and apoptosis[87].

The forkhead box protein O1 (foxo1), a master regulatory factor for gluconeogenesis and glycogenolysis, as well as a positive regulator of the expression of insulin and of a series of circulating proteins that are involved in glucose counterregulation, such as the insulin growth factor binding protein 1 (IGFBP1), and the retinol binding protein 4 (RBP4)[88].

foxo1 transcription factors affect a number of cell types that are important in the host response. Cell types whose functions are modulated by foxo1 include keratinocytes in the skin and mucosal dermis, neutrophils and macrophages, dendritic cells, Tregs and B-cells. foxo1 is activated by bacterial or cytokine stimulation. There are four members of this family in mammals, three of which (foxo1, foxo3, and foxo4) have conserved sequence homologies while foxo6 is more distantly related[89].

Foxos transcriptional activities are known to be regulated through the phosphorylation of their proteins by Akt, a kinase in the insulin signaling pathway, Thus, it is well established that the insulin-foxo pathway controls glucose and lipid metabolism [90].

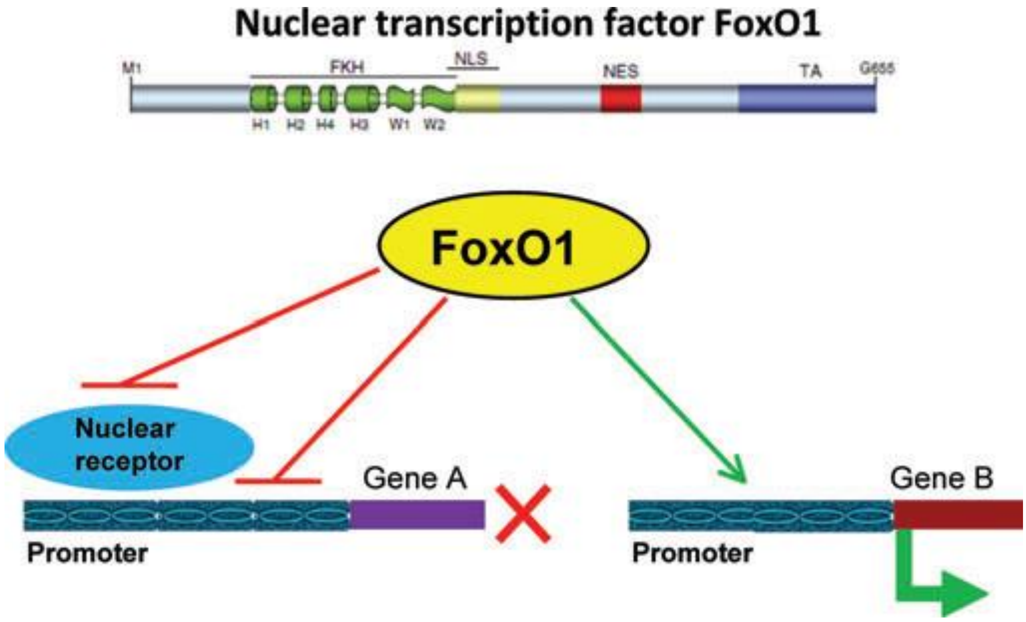


Figure 1.4 regulatory functions of nuclear transcription factor FoxO1. FoxO1 directly inhibits nuclear receptors like PPAR" or LXR. FoxO1 activates or represses the promoter of target genes [91].

1.5.1 Metabolic of Forkhead Transcription Factor1

Forkhead proteins, and FoxO1 in particular, play a significant role in regulating whole body energy metabolism. Glucose homeostasis is achieved by adjusting endogenous glucose production as well as glucose uptake by peripheral tissues in response to insulin. In the fasted state, the liver is primarily responsible for maintaining glucose levels, with FoxO1 playing a key role in promoting the expression of gluconeogenic enzymes. Following feeding, pancreatic beta cells secrete insulin, which promotes the uptake of glucose by peripheral tissues including skeletal muscle and adipose tissue, and can in part suppress gluconeogenic enzyme expression in the liver. In addition to directly regulating metabolism, FoxO1 also plays a role in the formation of both adipose tissue and skeletal muscle, two major organs that are critical for maintaining energy homeostasis. The importance of FoxO1 in energy homeostasis is particularly striking under conditions of metabolic dysfunction or insulin resistance[92].

1.5.1 FoxO1 Transcription Factor Role in Acne Vulgaris

Patients with acne, increasing the nuclear phosphorylation of foxo1 and their export into the cytoplasm, Nuclear deficiency and cytoplasmic expression of foxo1 are increased in the sebaceous glands of patients with acne compared to the healthy control group, [93].

FoxO1 is important of metabolism and cell proliferation, sebaceous gland hyperproliferation, lipogenesis, and hyperplasia of acroinfundibular keratinocytes, thereby contributing to the development of acne[94].

foxo1 has been proposed to function as a key regulator in the pathogenesis of acne as FoxO1, senses external nutrient and internal growth factor signals and relays these to foxo1-dependent gene regulation, Central to the regulation of foxos is their shuttling either into the nucleus or into the cytosol. foxo1 is inhibited by its export into the cytoplasm, which requires specific phosphorylation of foxo1in the nucleus by activated protein kinase(Akt) The phosphoinositol-3 kinase (PI3K)/Akt cascade is stimulated by growth factors like insulin and IGF-1 and is negatively regulated by the Phosphatase and tensin homolog PTEN[95].

Aim of the study

1. Evaluate the possible association between serum survivin and developing acne vulgaris and detect the relation of its levels with acne severity.
2. Determine the relationship between serum levels of FoxO1with the severity of acne vulgaris
3. Investigate the potential role of serum IGF-1 in acne vulgaris and severity of disease.

Chapter TWO

Materials and Methods

2. Materials and Methods

2.1. Study Design

The study was designed as a case-control study.

2.2. Materials

2.2.1. Chemical and kits

The chemicals and kits in the present study were used as supplied from purchases without additional purification. Kits and chemicals used in the present study are shown in Table 2-1.

Table 2-1 The Chemicals and Kits

No.	CHEMICALS	COMPANY AND COUNTRY
1	Human Insulin-like Growth Factors 1, IGF-1 ELISA Kit	**Bioassay (China)**
2	Human Survivin ELISA Kit	**Bioassay (China)**
3	Human Forkhead Box O1, FOXO1 ELISA Kit	**Bioassay (China)**

2.2.2. Instruments and Equipment

The instruments and equipment used in this study are listed in table 2-2.

Table 2-2 Instruments and Equipment's Used.

No.	Instruments & Equipment	Company & Country
1	Elisa washer, reader and printer	**Biotek /USA**
2	Deep Freezer	**Samsung/Korea**
3	Deionizer	**GFL/Germany**
4	Incubator	**Fisherclient./Germany**
5	Multichannel micropipette (0-250 µl)	**Slamed / Germany**
6	Micropipettes (5-50µl),(100-1000µl)	**Slamed / Germany**
7	Tank for multichannel micropipette	**China**
8	Eppindorf tube (0.5 ml)	**China**
9	Disposable syringe (5 ml)	**China**
10	1 ml pipette tips	**China**
11	0.1 ml pipette tips	**China**
12	Test tube with Separating gel	**AFCO , Jordan**
13	Centrifuge EBA 20	**ROTOFIX 32 A (Germany)**
14	Filter papers	**China**
15	Refrigerator	**agur/Turkish**

2.3. Subjects

2.3.1. The Place and Date of Study

This study was done in the laboratories College of Medicine, University of Babylon and the samples were collected from the registered attendances of the dermatology Clinic /Al-Musayyib General Hospital, Al-Imam Al-Sadiq Hospital, Murjan Teaching Hospital during the period from 1st of January 2022 until 1st of September 2022. and the patient were diagnosed by specialist dermatologist

2.3.2. Patients Group

The sample size of the patient group in the present study was selected according to Daniel's sample size formulae equation[96]. consist of 45 patients (25 male and 20 female).

2.3.3 The Diagnosis of Patients Group

In this study, the diagnosis of acne vulgaris patients was confirmed by a dermatologist detailed questionnaire was filled out, that contains (age, gender, weight, height).

2.3.4 Control Group

A sample of control group of 45 healthy individuals (25 men and 20 females) was collected . were matched with patients in terms of gender and age ,They were obtained from health volunteer without any history of AV.

2.3.5. Exclusion Criteria

Diabetes patients, hypertention , menstrual disorders, obesity, thyroid diseases , poly cystic ovary, smoking, treated patients.

2.3.6 Inclusion Criteria

Participations were proven with acne vulgaris within the age from (15 – 30) years. Mean age (21.27 ± 3.19) were in the current study as a patient group, in addition to apparently healthy individuals as control group within the age from (15-30) years mean age (23.22 ± 3.42).

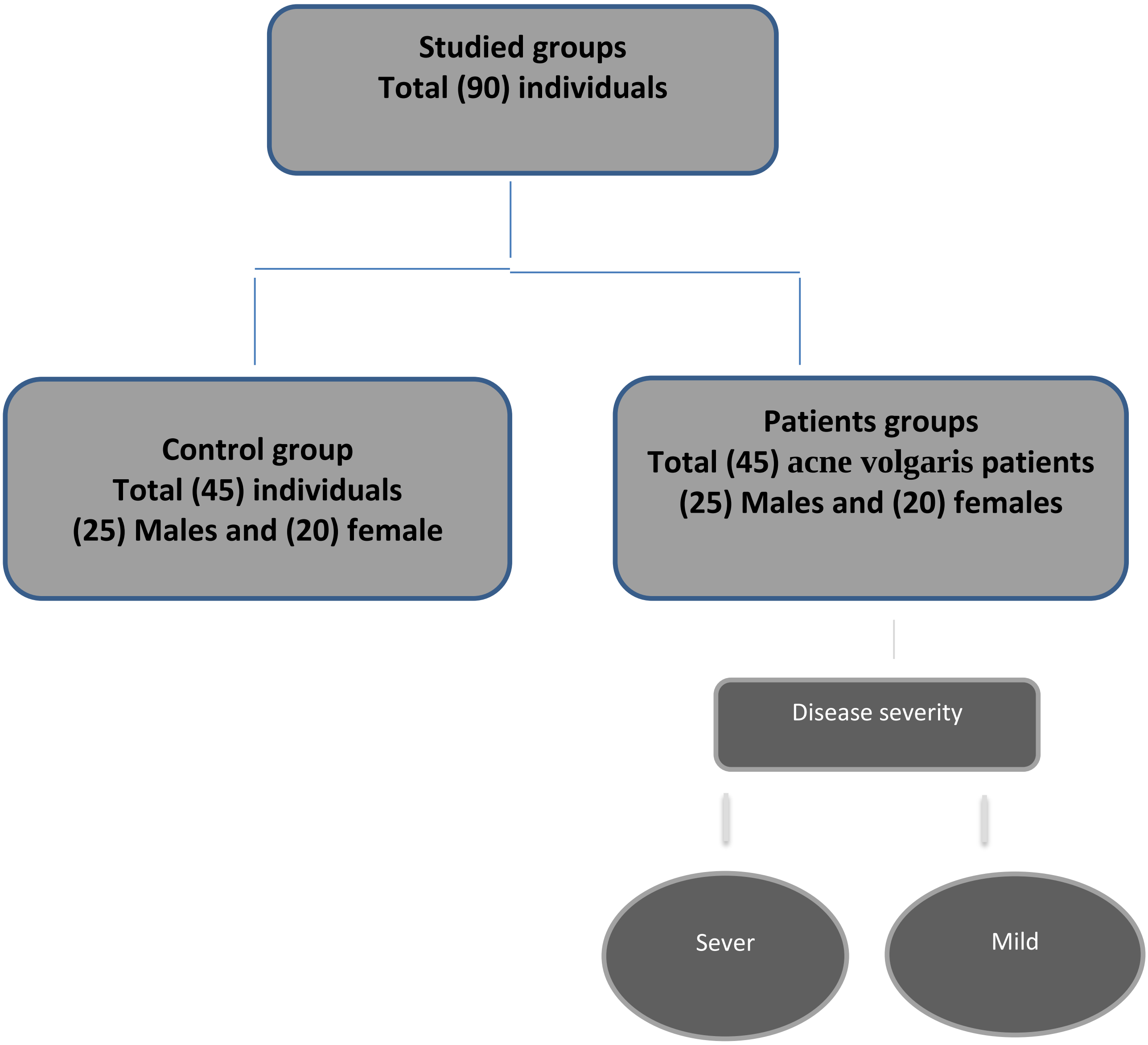


Figure 2-1 studied groups

2.3.7. Ethical Issues

Depends on the following:

1- Approval by a scientific committee College of medicine (University of Babylon, Iraq) and the Biochemistry Department in the same college.

2- The aims and procedure of this study were clarified to all participants in the present study to gain their verbal acceptance

3. Health department approval

2.4. Methods

2.4.1. Collection of Blood Sample

A blood sample was collected from the vein of each participant using a needle puncture approximately (5ml) was placed in disposable tubes containing separating gel, The blood sample in the gel tube was kept at room temperature for five minutes to coagulate. And later it centrifuged for (10 minutes) at 3000× g subsequently, then pipetting the transparent serum into three clear dry eppendorf tubes and stored at (-20 C) until used for the several examinations. The serum was thawed at (20-25 C) temperature for 1 hours then submitted to the centrifuge for 5 minute at 3600 g.

2.4.2. Body Mass Index (BMI)

Body mass index was measured in all individuals based on a weight-to square height ratio derived by achieving a mathematical equation that divided the weight in kilograms by the square height in meters[97]. the results were considered as follows:

1. Underweight ≤ (18.5)
2. Normal weight (18.5 - 24.9)

3. Overweight (25-29.9)
4. Obese ≥ (30)

2.5. Human Insulin like Growth Factors 1 IGF-1 ELISA Kit

2.5.1. Measurement Human Insulin-like Growth Factors 1 IGF-1 ELISA Kit

2.5.2. Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IGF1 antibody. IGF1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IGF1 Antibody is added and binds to IGF1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IGF1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IGF1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Table 2-3 Components of Human Insulin-like Growth Factors 1 IGF-1 ELISA Kit

Components	Quantity (96T)
Standard solution (48ng/ml)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human IGF1 antibody	1ml x1
User instruction	1
Plate sealer	2 pics

2.5.3 Reagent preparation

.A. All reagents has been put brought to room temperature before use.

B. The original standard sample was diluted as the following table:

24ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
12ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
6ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
3ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
1.5ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

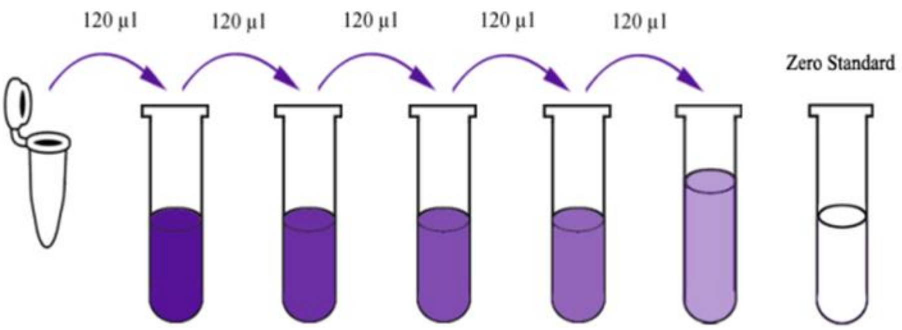


Figure 2-2 Concentration of standards of Human Insulin-like Growth Factors 1 IGF-1 ELISA Kit

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
48ng/ml	24ng/ml	12ng/ml	6ng/ml	3ng/ml	1.5ng/ml

C. wash buffer : diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

2.5.4 .Procedure:

1. Prepare All reagents, standard solutions and samples were brought to room temperature before use. The assay is performed at room temperature.

2. It has been selected the number of strips required for the assay. The strips have been inserted in the frames for use. The strips unused are stored the at 2-8°C.

3. A volume of 50µl standard was added to standard well. Note: The antibody is not added to the standard well because the standard solution contains biotinylated antibody.

4. volume of 40µl sample was added to sample wells and then added 10ul Human IGF1 antibody to sample wells, then added 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealant. and incubated for 60 minutes at 37°C.

5. The sealer has been removed from well of IGF-1 and washed the plate 5 times with wash buffer. Wells have been drained with 300µl , washed buffer for 30 seconds to 1 minute for each wash. For automated washing, has been aspirate or decant each well and washed 5 times with wash buffer. the plate has been bloted onto paper towels or other absorbent material.6. volume of 50µl substrate solution A was added to each well and then added 50µl substrate

solution B to each well. The plate is incubated and covered with anew sealer for 10 minutes at 37°C in the dark.

7. A volume of 50µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density has been determined (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution[97].

2.5.5 Calculation of Results

Construction complete a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

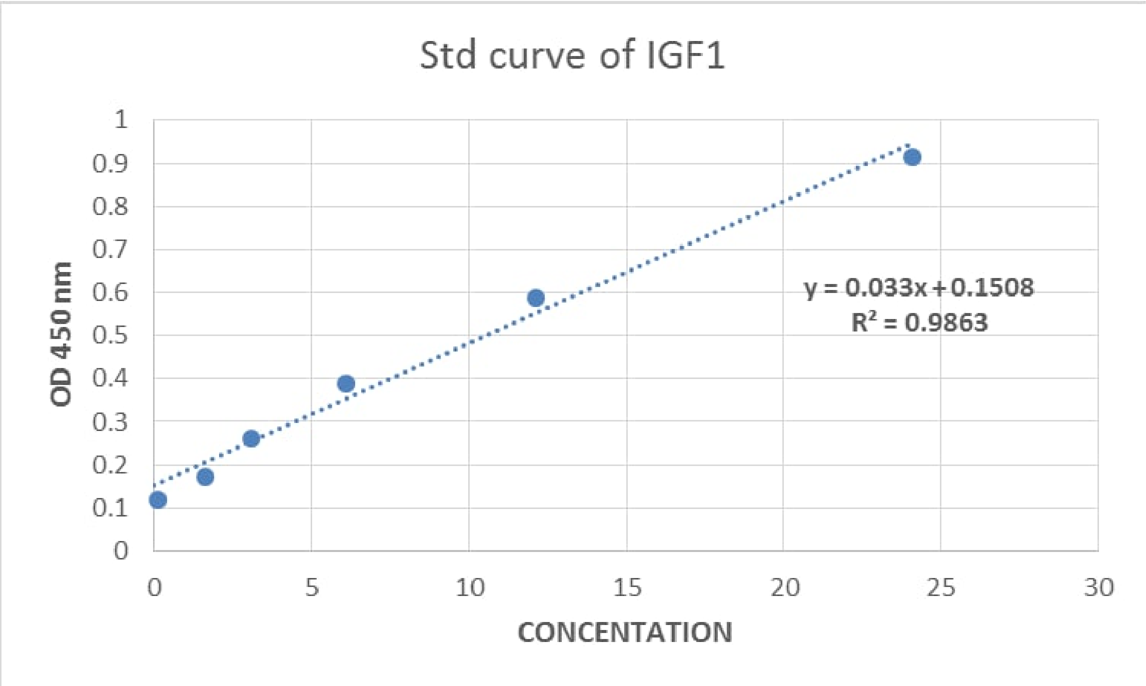


Figure 2.3 standard curve of IGF1

2.6 Human Survivin ELISA Kit

2.6.1 Measurement Human Survivin ELISA Kit

2.6.2 Principle

This kit is an Enzyme-Linked Immuno sorbent Assay (ELISA). The plate has been pre-coated with Human Survivinantibody. Survivin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human Survivin Antibody is added and binds to Survivin in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated Survivin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human Survivin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Table 2-4 Components of Human Survivin ELISA Kit

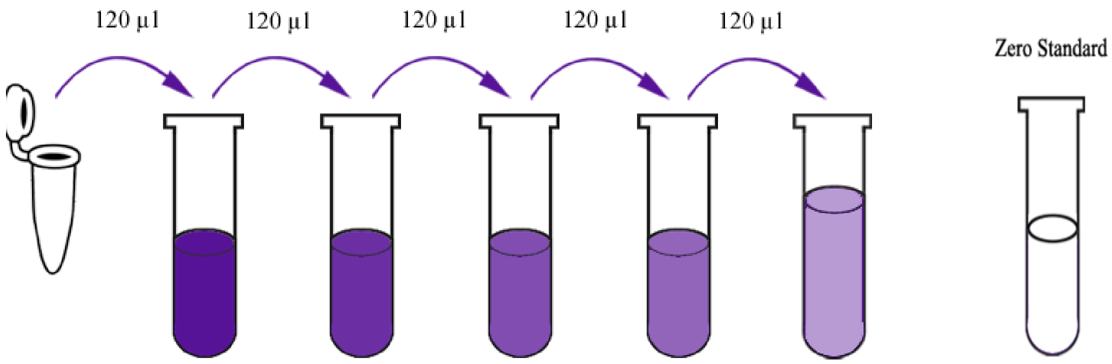
Components	Quantity
Standard solution (1280 ng/L)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human Survivin Antibody	1ml x1
User instruction	1
Plate sealer	2 pics
Zipper bag	1pic

2.6.3 Reagent preparation

A. All reagents has been put brought to room temperature before use.

B. The original standard sample was diluted as the following Table:

640ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
320ng/L	Standard No.4	120µl Standard No.5 + 120ul Standard diluent
160ng/L	Standard No.3	120µl Standard No.4 + 120ul Standard diluent
80ng/L	Standard No.2	120µl Standard No.3 + 120ul Standard diluent
40ng/L	Standard No.1	120µl Standard No.2 + 120ul Standard diluent



Figure(2-4): Concentration of standards of Human Survivin ELISA Kit

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1280ng/L	640ng/L	320ng/L	160ng/L	80ng/l	40ng/L

C. wash buffer : diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

2.6.4. Procedure

1. Prepare All reagents, standard solutions and samples were brought to room temperature before use. The assay is performed at room temperature.

2. It has been selected the number of strips required for the assay. the strips have been inserted in the frames for use. The strips unused are stored the at 2-8°C.

3. A volume of 50µl standard was added to standard well. **Note**: the antibody is not added to the standard well because the standard solution contains biotinylated antibody.

4. A volume of 40µl sample was added to sample wells and then added 10µl anti-Survivin antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. The plate was covered with a sealant and incubated for 60 minutes at 37°C.

5. The sealer has been removed from survivin and washed the plate 5 times with wash buffer. wells have been seale with at least 0.35 ml washed buffer for 30 seconds to 1 minute for each wash. For automated washing, has been aspirate all wells and washed 5 times with wash buffer, overfilling wells with wash buffer. the plate has been bloted onto paper towels or other absorbent material.

6. A volume of 50µl substrate solution A was added to each well and then added 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°Cin the dark.

7. A volume of 50µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density has been determined (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution. [98].

2.6.5 Calculation of Results

Construction complete a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

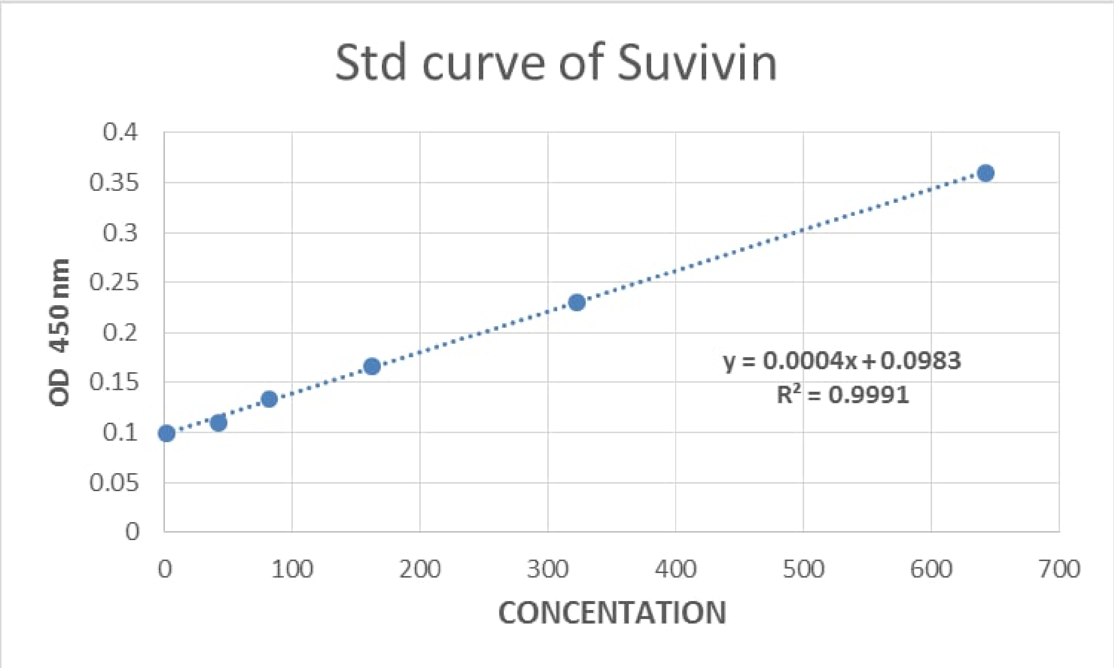


Figure 2.5 standard curve of survivin

2.7. Human Forkhead Box O1, FOXO1 ELISA Kit

2.7.1 Measurement Human Forkhead Box O1, FOXO1 ELISA Kit

2.7.2. Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human FOXO1 antibody. FOXO1 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human FOXO1 Antibody is added and binds to FOXO1 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated FOXO1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human FOXO1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Table 2-5 Components of Human Forkhead Box O1, FOXO1 ELISA Kit

Components	Quantity (96T)
Standard solution (24ng/ml)	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human TNFA antibody	1ml x1
User instruction	1
Plate sealer	2 pics

2.7.3.Reagent preparation

A-All reagents should be brought to room temperature before use.

B-The original standard sample was diluted as the following Table:

12ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
6ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
3ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
1.5ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
0.75ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

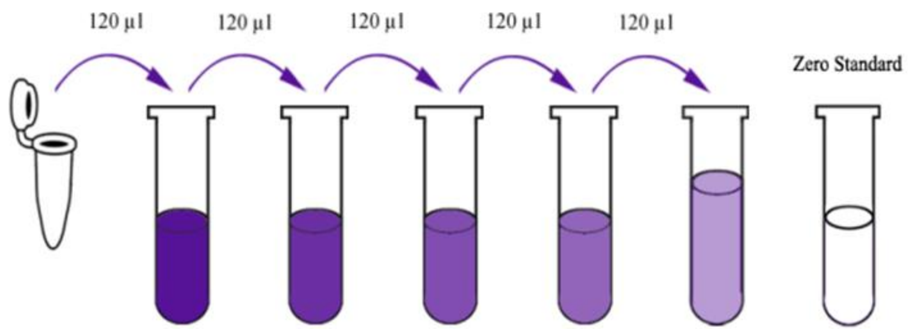


Figure 2-6 Concentration of standards of Human Forkhead Box O1, FOXO1 ELISA Kit

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
24/ml	12ng/ml	6ng/ml	3ng/ml	1.5ng/Lml	0.75ng/ml

C-Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2.7.4 Procedure

1. Prepare all reagents, standard solutions and samples were brought to room temperature before use. The assay is performed at room temperature.

2. It has been selected the number of strips required for the assay. The strips has been inserted in the frames for use. The strips unused are stored the at 2-8°C.

3. A volume of 50µl standard was added to standard well. Note: the antibody is not added to standard well because the standard solution contains biotinylated antibody.

4. A volume of 40µl sample was added to sample wells and then added 10ul Human FOXO1 antibody to sample wells, then added 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mixed well. the plate was covered with a sealant. Incubated for 60 minutes at 37°C.

5. The sealer has been removed and washed the plate 5 times with wash buffer. wells have been drained with 300µl washed buffer for 30 seconds to 1 minute for each wash. For automated washing, has been aspirate or decant each well and washed 5 times with wash buffer. the plate has been bloted onto paper towels or other absorbent material.

6. A volume of 50µl substrate solution A was added to each well and then added 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. A volume of 50µl Stop Solution was added to each well, the blue color will change into yellow immediately.

8. The optical density has been determined (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution[99].

2.5.7 Calculation of Result

Construction complete a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

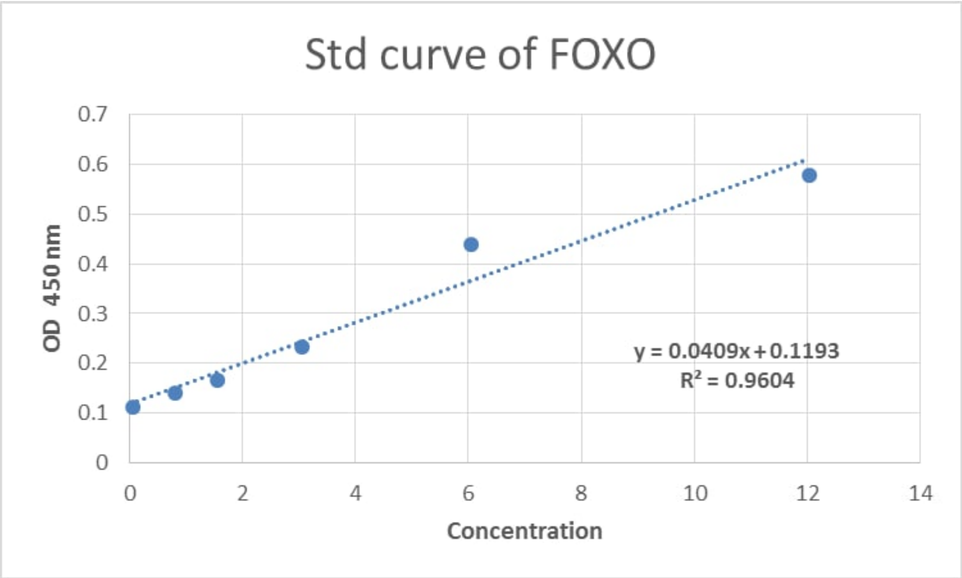


Figure 2.7 Standard Curve of Foxo1

2.8 Statistical Analysis

Statistical analysis was carried out using SPSS version 28. Continuous variables were given as (Mean SD) while categorical variables were provided as frequencies and percentages. Student t-test was used to compare means between two groups and the correlation test (Pearson test) was performed to find the correlation between variables. P-value less than 0.05 was considered as significant.

Chapter Three

The Results and Discussion

3. The Results and Discussion

3.1 Demographic Characteristics in Patients and Control

3.1.1 Examination the Age and BMI

In this study, 90 participants were divided into 45 patients and 45 healthy people, and their ages ranged between (15-30) years. A comparison between study groups regarding age and BMI was performed using a t-test. there was not statistically significant difference p value for age is (0.71),and BMI is (0.56).

The results presented in Table (3-1) for age and BMI showed that there was no significant difference (P value >0.05). This matching is important to eliminate any effects on the results that may arise from the difference in these characteristics.

Risk factors that showed significant differences between acne cases and controls

A positive familial history of acne was strongly associated with an increased risk for acne presentation, even after controlling for age and BMI , smoking status,

Out of the evaluated lifestyle factors, computer/TV usage had significant association with acne presentation, while presence of household smokers and alcohol consumption were significantly associated with acne severity extent. computer/TV usage was used as an indication of a sedentary lifestyle, thus this finding suggests that acne is more common among those with more sedentary lifestyles. A previous study also reported a lower risk of acne in those who used the computer for under 2 h per day relative to those with higher computer us age , the influence of smoking status on acne presentation and severity is controversial, with inconclusive evidence on whether smoking is a protective or risk factor[100].

Table 3-1: The mean differences of age and BMI according to study group

variable	group	N	Mean ± SD	P –value
AGE(year)	control	45	23.22 ± 3.42	0.71
	patient	45	21.27 ± 3.19	
BMI(kg/m^2)	control	45	23.26 ± 2.44	0.56
	patient	45	22.92 ± 3.12	

P value ≤ 0.05 was significant

The distribution of patients according to BMI shown in Figure 3-1

Majority of patients (64 %) were normal weight

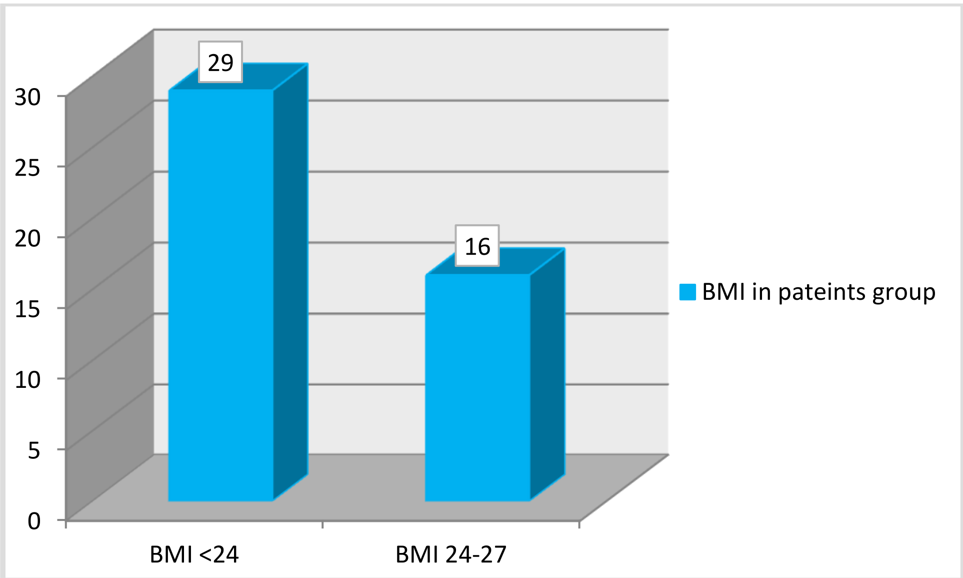


Figure 3-1: The Distribution of patients according to BMI.

3-1-2.Examination the Gender of the Study Groups

The gender distribution of the studied groups was 45 patients with acne vulgaris, 25 (56.00%) male and 20 (44.00%) female, matching with controls and the results represented in figure 3.2.

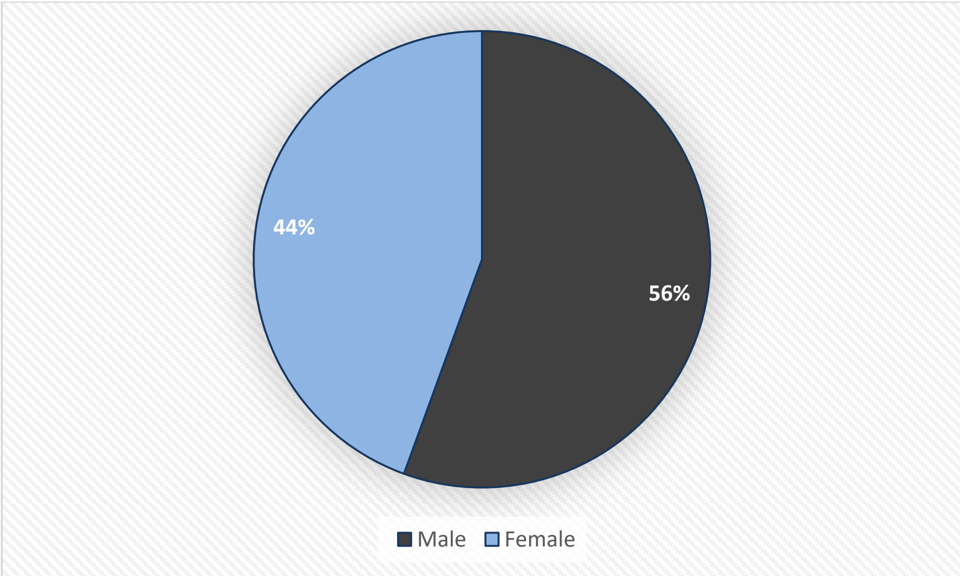


Figure 3.2: The Distribution of patients according to Gender

3-2. Examination the Effect of Biochemical Parameters In the Study Groups

The findings demonstrated significant difference in comparison between study groups of IGF-1, survivin and foxo1,as shown in Table 3-2

Table 3-2: Comparison of parameters IGF1, Survivin, and Foxo1 in patients and control groups.

Parameter	Group	N	Mean ± SD	p-value
IGF1(ng\ml)	control	45	3.95 ± 2.88	**0.003 ***
	patient	45	7.12 ± 4.61	
Survivin(ng\l)	control	45	173.03 ± 87.62	**<0.001 ***
	patient	45	243.13 ± 75.29	
Foxo1(ng\ml)	control	45	2.37 ± 1.57	**0.001 ***
	patient	45	4.74 ± 3.4	

P value ≤ 0.05 was significant

The current study show that been a greater significant difference in IGF1,survivin and foxo1 between AV patients and healthy control ($P \leq 0.05$). As shown in Table 3.3

This study showed that the mean and SD of the IGF1,Survivin and Foxo1 for patient was (7.12 ± 4. 61, 243.13± 75.29, 4.74±3.4) and for healthy control was (3.95±2.88,173.03±87.62, 2.37±1.57) respectively.

IGF-1 can suppress foxo1 nuclei in SZ95 sebocytes which play a role in increasing lipogenesis ,foxo1 functions as a regulator in the pathogenesis of AV where foxo1 integrates external and internal growth factors signals at the level of gene[101].

3.2.1 Study The Effect of Insulin Like Growth Factort1 (IGF1)

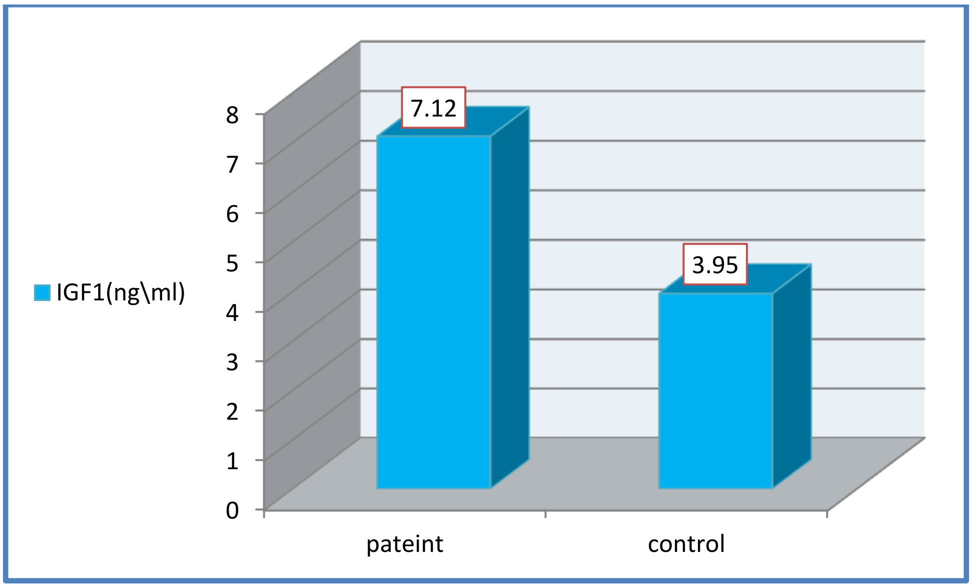


Figure (3-3) show comparison between patient and control in IGF1.

A significantly higher serum IGF-1 level was found in the patients with acne than in the controls. Excess consumption of a high-glycaemic-load diet was significantly associated with higher serum levels of IGF-1 and cytoplasmic expression of foxo1 and mTOR[102]. foxo1 is also a negative regulator of growth hormone receptor (GHR), which plays a role in hepatic IGF-1 synthesis. Thus, insulin signaling represses foxo1, stimulating IGF-1 synthesis. Sebum IGF-1 concentrations, in turn, it has been demonstrated to correlate linearly with sebum excretion rates in male patients, and increased sebum IGF-1 levels have been observed in women with post-adolescent acne.

Furthermore patients observing an aggravation of acne by food intake have exhibited higher IGF-1 serum levels compared to patients who do not observe an aggravation of acne by food intake[103].

Evaluation of serum levels of Insulin Like Growth Factor-1(IGF-1) in seborrheic dermatitis patients. Serum IGF-1 concentrations were measured in 50 patients with SD and 30 healthy controls using enzyme-linked immunosorbent assay (ELISA). Serum levels of IGF-1 in patients with SD were found to be higher than in healthy controls (P =0.045). Increased serum IGF-1 levels probably reflect the role of IGF-1 in the pathogenesis of SD[104].That agrees with the current study results

3.2.2 Study The Effect of Survivin on The Study Groups

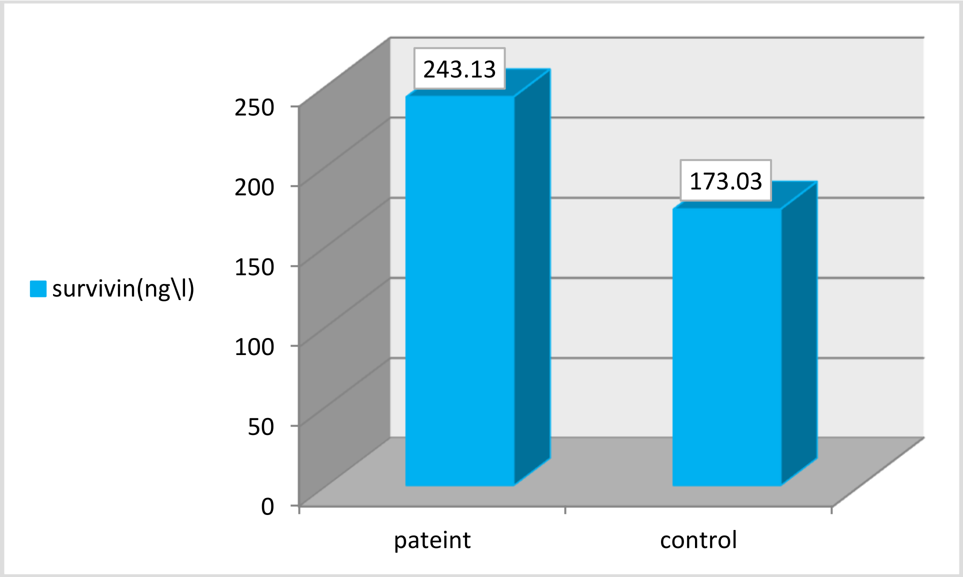


Figure (3-4) difference in survivin levels in AV patient and control.

Survivin, a member of inhibitors of the apoptosis (IAP) gene family, that inhibits apoptosis and regulates cell division, proliferation, and survival, Furthermore, other study showed that serum survivin levels were significantly increased in active acne ($P<0.05$) and acne scar ($P<0.001$) groups in comparison to the healthy control group[105]. That agrees with the current study results.

3.2.3 FOXO1

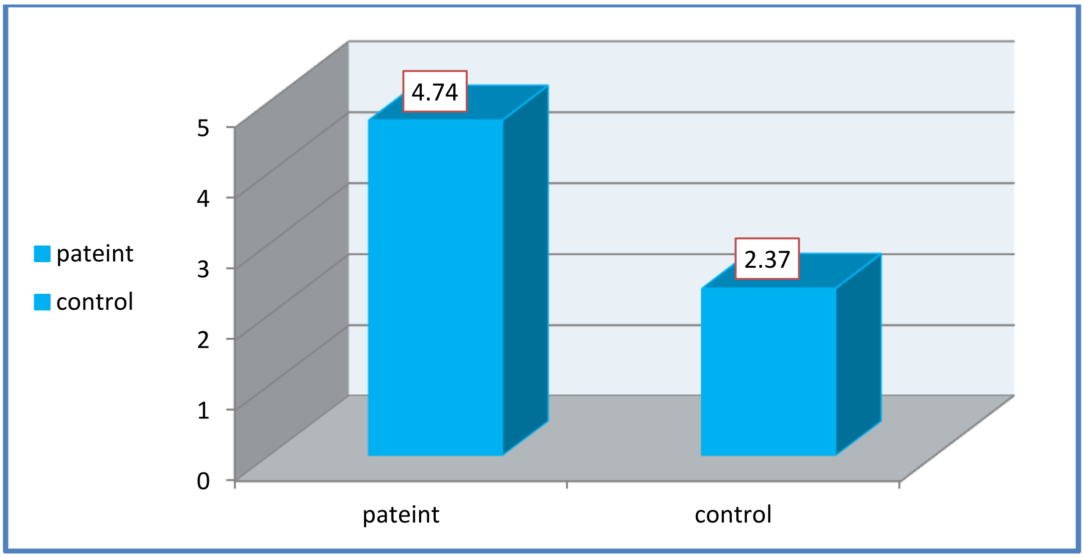


Figure 3-5 FOXO1 levels in acne vulgaris patient and control

all major factors of acne pathogenesis, i.e. androgen-mediated signal transduction, comedogenics, increased sebaceous lipogenesis , and follicular, are related to a nuclear increased of the forkhead box O transcription factor foxo1. another study showed an increase in the expression of foxo1 in acne patients compared to healthy controls[106].

3.2.4 Comparison of Parameters According to Gender Subgroup.

The results show significant difference in level of survivin between male and female but no significant difference in level of IGF-1 and Foxo1 between this subgroup, as shown in Table 3-3

Table 3-3: Comparison of parameters (IGF),(survivin),and (Foxo1) According to Gender Subgroup.

Parameter	Gender subgroup	N	Mean ± SD	p-value
IGF1(ng\ml)	male	25	6.32 ± 4.47	0.26
	Female	20	8.86 ± 5.10	
survivin(ng\l)	male	25	219.5 ± 55.3	**0.017** *
	Female	20	272.6 ± 87.2	
foxo1(ng\ml)	male	25	3.88 ± 2.4	0.13
	Female	20	5.82 ± 4.6	

The serum survivin levels were statistically significant were compared according to gender, females had higher levels of survivin than the males (272.6±87.2 versus 219.5±55.3 ng/L), p value is (0.017).

When the serum survivin levels were compared according to gender, females had higher levels of survivin than the males [107]. That agrees with the current study results

The results were shown no significant difference in level of IGF-1, survivin, and Foxo1 between BMI subgroup, as shown in Table 3.4

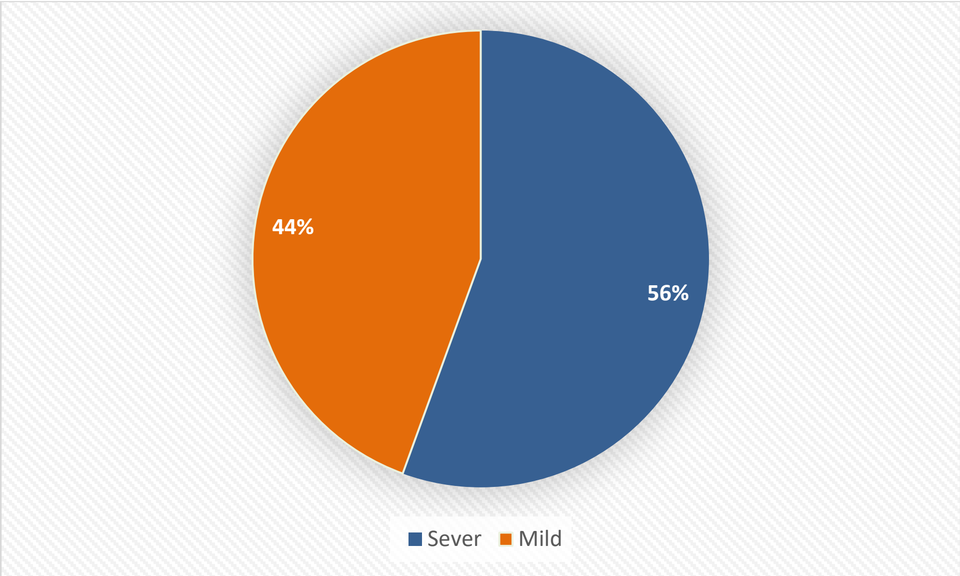
Table 3.4: Comparison of Parameters (IGF1),(survivin),and (Foxo1) According to BMI Subgroup of Patients.

Parameter	BMI sub group	N	Mean ± SD	p-value
IGF1(ng\ml)	normal weight	29	7.13 ± 5.01	0.58
	over weight	16	5.84 ± 4.17	
survivin(ng\l)	normal weight	29	241.1 ± 82.4	0.81
	over weight	16	246.7 ± 62.5	
foxo1(ng\ml)	normal weight	29	4.98 ± 3.8	0.62
	over weight	16	4.31 ± 3.9	

There was no significant association between the Acne Vulgaris and BMI. which was not significantly associated with BMI. [108]. Overweight and obesity are associated with acne in female aged 18 and 19, but the same association was not observed in male . However, found no significant association between increased BMI and AV[109]. That agrees with the current study results

3.2.5. Study the Effect of biomarkers on the Study Groups Based on the Severity of Disease

In severe acne, nodules, and cysts can cause scarring and psychological effect. It usually affects the face, shoulders, and upper trunk. It can lead to substantial psychological distress, including low self-esteem, depression, and anxiety, despite being a common disease treated by a physician, there is no ideal objective assessment method, Numerous methods have been used, some of these methods depend on photographs representing different grades of severity, some of them depends on the text description of lesions[110].



3.6 Figure Severity of Disease

Table 3.5: Comparison of Parameters (IGF), (survivin),and (Foxo1) According to Severity of Disease

The results show no significant difference in level of IGF-1, and foxo1 between mild and sever cases of AV, and significant in survivin as shown in Table 3.5

Parameter	subgroup	N	Mean ± SD	p-value
IGF1(ng\ml)	Mild	20	5.13 ± 4.09	0.21
	Sever	25	7.37 ± 5.82	
survivin(ng\l)	Mild	20	223.9 ± 58.1	0.04
	Sever	25	271.0 ± 85.6	
foxo1(ng\ml)	Mild	20	4.1 ± 3.7	0.42
	Sever	25	5.2 ± 4.6	

in other study show there was no significant association found between the IGF1,foxo1 and severity of acne[111]. another study showed that there is significant association between the severity of acne and survivin [112]. survivin, is increase with increasing degrees of the severity of AV[99].that agrees with the current study results.

3.3. Study the Correlation between The biomarkers

This table showed correlation between BMI , Age ,IGF1, survivin ,foxo1

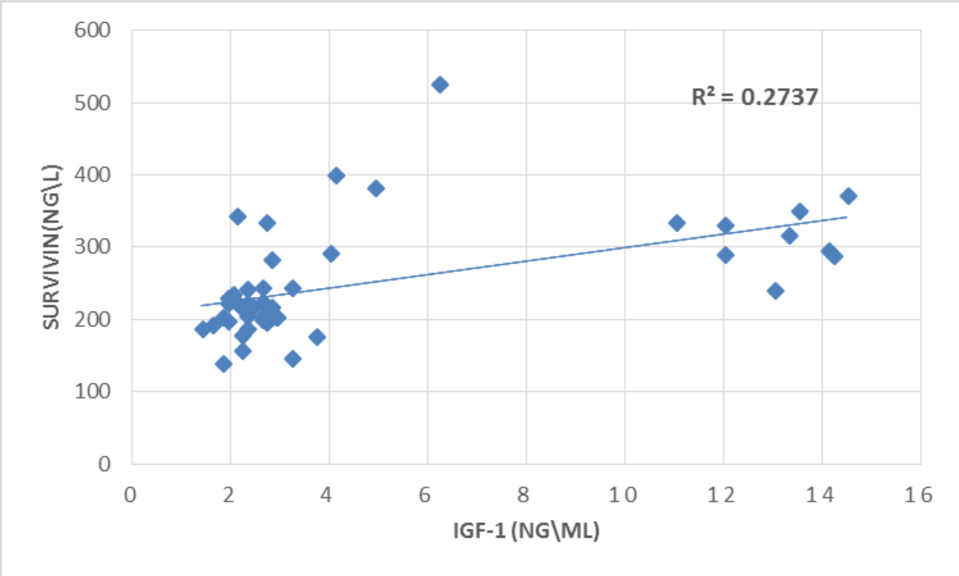
Table 3-6: correlation between all parameters

		BMI	AGE	IGF1	survivn	foxo1
BMI	r	1	.017	-.012-	.127	-.024 -
	P-value		.913	.936	.404	.877
	N	45	45	45	45	45
AGE	r	.017	1	.338	.470	.336
	P-value	.0.913		.23	.08	.07
	N	45	45	45	45	45
IGF1	r	-.012-	.338	1	.568	.877
	P-value	.936	.23		**.000**	**.000**
	N	45	45	45	45	45
survivin	r	.127	.470	.568	1	.524
	P-value	.404	.08	.000		**.000**
	N	45	45	45	45	45
foxo1	r	-.024 -	.336	.877	.524	1
	P-value	.877	.07	.000	.000	
	N	45	45	45	45	45

In our study there were no correlation between BMI and Age studied parameter, where p value is (>0.05)

3.3.1 Study the Correlation Between IGF1 And Survivin

There were positive correlation between IGF1and survivin, where p value is (<0.05)

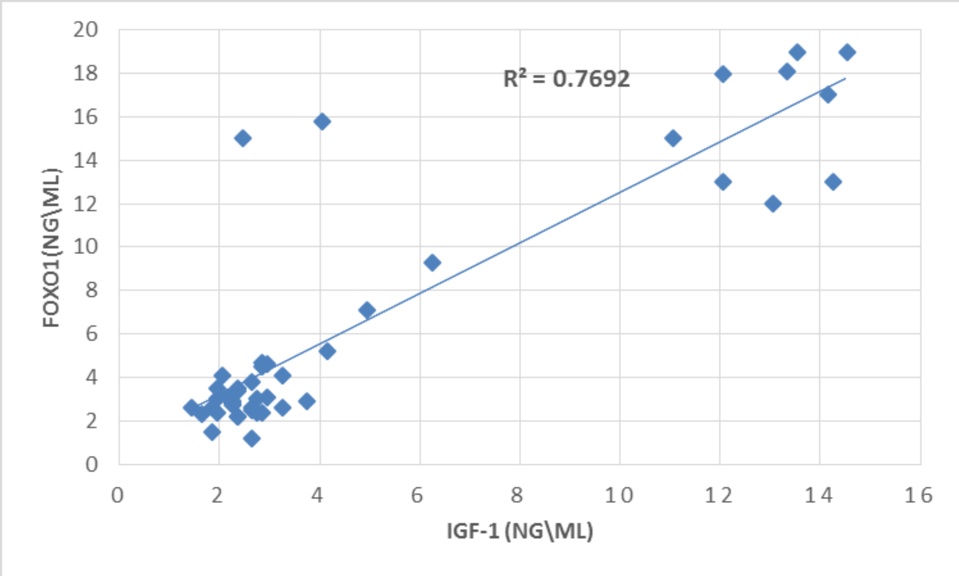


Our findings suggest that IGF-I and survivin could play potential roles in the pathogenesis of active acne vulgaris and more importantly in postinflammatory acne scars with significant positive correlation coefficient between serum levels of IGF-I and survivin which support IGF-I-/PI3K-/AKT-mediated.

The results of the current study showed that serum levels of IGF-I were significantly elevated in active acne (p<0.01) and acne scar (p<0.001) groups in comparison to the healthy control group. Also, IGF-I serum levels showed significant increase in acne scar group compared to active acne group (p<0.05)[103] . in other study there was a positive correlation between survivin and serum IGF-I levels in patients with acne [113].

3.3.2 Study the Correlation Between IGF1 And Foxo1

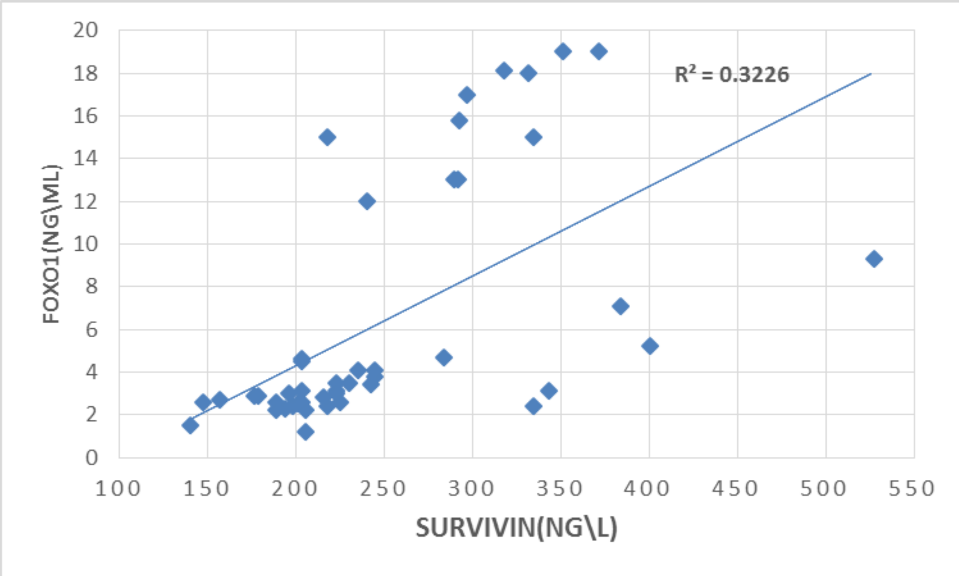
There were positive correlation between IGF1 and Foxo1, where p value is (<0.05)



Several studies have reported that insulin-like growth factor (IGF)-1, forkhead box transcription factor foxo1 and interactions may be the key to understanding the links between genetic and environmental factors in acne vulgaris , a significantly higher serum IGF-1 level was found in the patients with acne than in the controls. The cytoplasmic expression of foxo1 was found to be significantly greater in the acne group, whereas in the control subjects this expression was likely to be nuclear, Excess consumption of a high-glycaemic-load diet was significantly associated with higher serum levels of IGF-1 and cytoplasmic expression of foxo1[100].

3.3.3 Study the Correlation Between Survivin And Foxo1

There were positive correlation between survivin and foxo1, where p value is (<0.05)



Several studies have shown that forkhead box Class O1 foxo1, survivin, play a role in the pathogenesis of multifactorial AV. statistical test results showed a significant correlation of foxo1, survivin levels, (p < 0.05)[99].

3.4 ROC Curve of Biochemical Parameters.

3.4.1 ROC Analysis of Survivin.

ROC curve for the sensitivity and specificity of Survivin (ng\l) for diagnosis of acne cases compare with control , (Cut-off point was ≥ 198.1(ng\l) , AUC=0.82 , P= 0.001*, the sensitivity and the specificity was 84.4 %, 77.8 % respectively

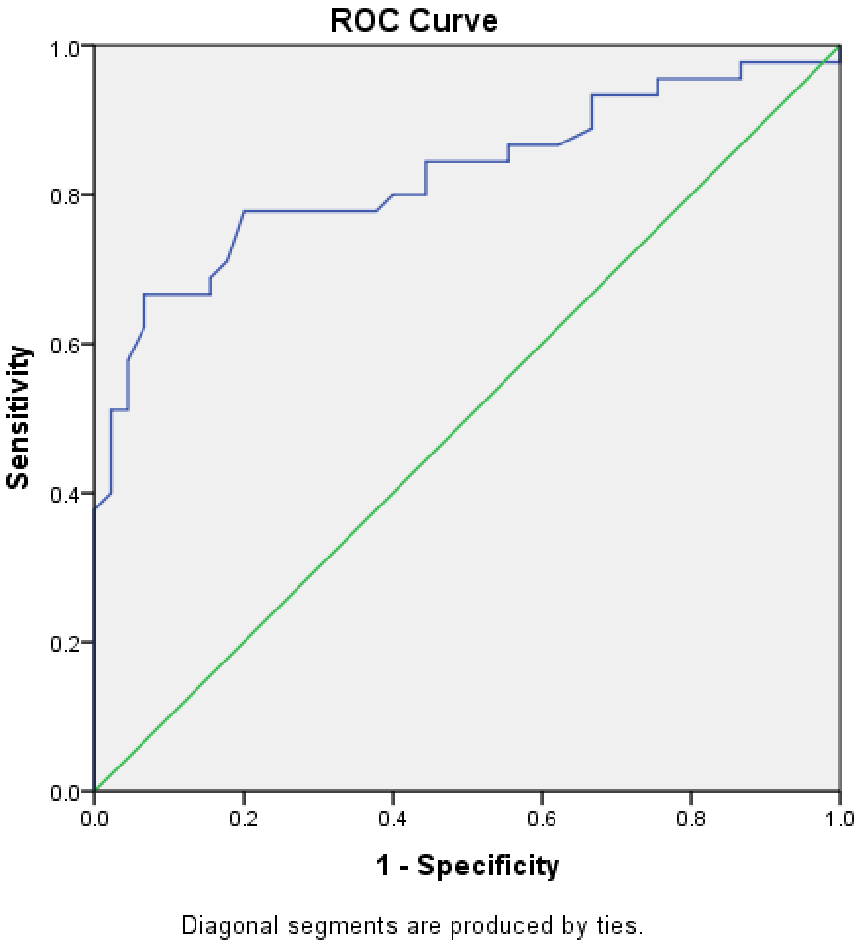


Figure 3.7 ROC Curve For Survivin

in other study elevated serum levels of survivin were determined in acne vulgaris[114]. that agrees with the current study results

3.4.2 ROC Analysis of FOXO 1.

ROC curve for the sensitivity and specificity of FOXO 1 (ng\ml) for diagnosis of acne cases compare with control (Cut-off point was ≥ 2.98 (ng\ml) , AUC=0.76, P= <0.001*, the sensitivity and the specificity was 77.8 %, 60.2 % respectively

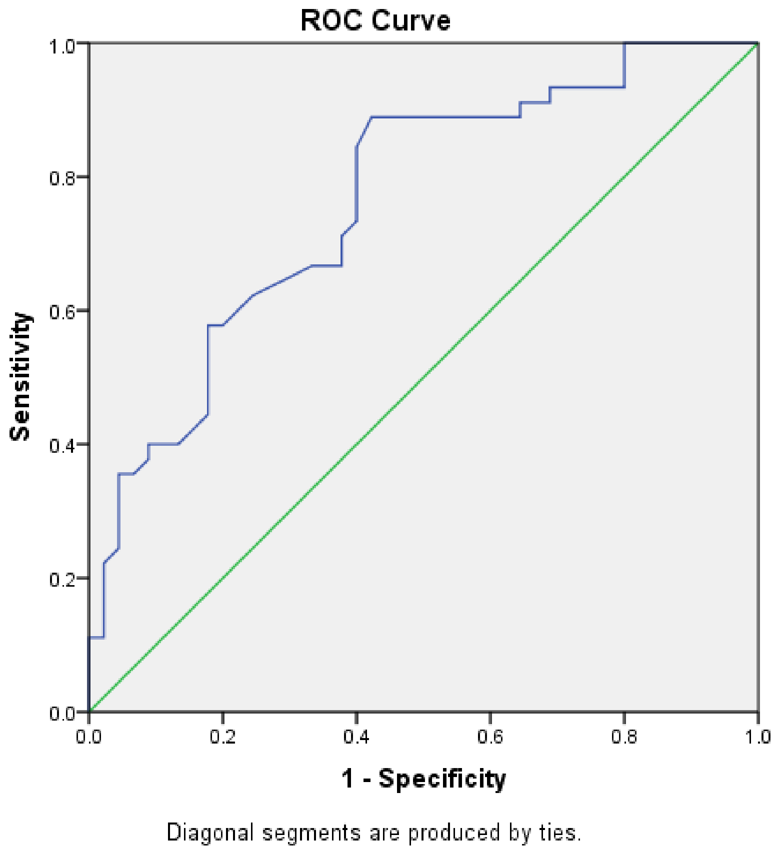


Figure 3.8 ROC Curve For Foxo1

other studied the cytoplasmic expression of foxo1 and it was found to be significantly greater in patients than control[115]. that agrees with the current study results

Conclusions and Recommendations

Conclusions:

1. This study collectively demonstrate that both IGF-I , survivin and foxo1 play an important role in the pathogenesis of active acne vulgaris.
2. Both IGF-I ,survivin had more importantly role in the pathogenesis of postinflammatory acne scar fibrotic tissue formation.
3. There is no relationship between foxo1 and IGF-1 in severity of acne, high level of survivin was correlated with severity of acne vulgaris .

Recommendations:

1. Additional more comprehensive large-scale studies on the expression and subcellular localization of IGF-I , survivin and foxo1 are recommended to determine their correlation with the severity of these dermatological ailments.
2. Future studies should clarify whether sebaceous gland IGF-I, survivin and foxo1 are upregulated in sebaceous cell hyperplasia of acne vulgaris.
3. Determine whether isotretinoin treatment downregulates IGF-I, survivin expression and foxo1.
4. It is recommended that topical metformin be used.

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الخلاصة

حب الشباب الشائع وهو اضطراب جلدي التهابي مزمن ذو الية إمراضية معقدة, هو ثامن أكثر الأمراض شيوعًا في جميع أنحاء العالم. يحدث حب الشباب الشائع على خلفية الزهم الدهني وهو مرض محدد وراثيًا يتميز بزيادة إفراز الغدد الدهنية المتغيرة كيميائيًا وظهور حب الشباب في مناطق الجلد الغنية بالغدد الدهنية

عادة ما يظهر المريض المصاب بحب الشباب بتاريخ من الحطاطة ، البثرات الأكثر شيوعًا التي تصيب الوجه والظهر والصدر والكتفين. غالبًا ما تكون الأعراض الجهازية غائبة ، ولكن قد يصف المريض الأعراض الموضعية للألموالاحمرار

العوامل الذات صله في حب الشباب هي عامل النمو الشبيه بالأنسولين -1 ، وسيرفايفين وعامل النسخ.

الهدف من الدراسة هو التحقيق من العلاقة بين مستويات عامل النمو الشبيه بالأنسولين -1 وسيرفايفين وعامل النسخ لدى مرضى حب الشباب والاصحاء

تم تصميم هذه الدراسة كدراسة حالة وضبط. شارك هذه الدراسة 45 مريضًا بحب الشباب (20 إناث و 25 ذكورًا) بالإضافة إلى 45 شخصًا سليمًا (20 إناثًا و 25 ذكرًا) متطابقين جيدًا مع المرضى في العمر والجنس. كان العمر (15 - 30) سنة متوسط العمر 21.27 ± 3.19 سنة ,مؤشر كتلة الجسم (22.92 ± 3.12) كغم / م 2 ، تم أخذ عينات من عيادات الأمراض الجلدية في مستشفى المسيب العام ومستشفى المرجان التعليمي ومستشفى الامام صادق (ع)

تم قياس مستويات عامل النمو الشبيه بالأنسولين -1 وسيرفايفين وعامل النسخ
أظهرت نتائج الاختبارات التي أجريت أن مستوياتهم كانت أعلى بكثير من الأشخاص الأصحاء حيث بلغت قيمة

(P=<0.05)

أظهرت العلاقة ارتباطًا كبيرًا بين مستوى سيرفايفين من ناحية الجنس حيث كان مستواه في الاناث اعلى من مستواه في الذكور كانت قيمة

.(P=<0.0 17)

عدم وجود ارتباط كبير بين مستوى عامل النمو الشبيه بالأنسولين -1 وسيرفايفين وعامل النسخ وبين مؤشر كتلة الجسم وكذلك عدم وجود ارتباط بين عامل النمو الشبيه بالانسولين وعامل النسخ وبين شدة حب الشباب الى انه وجد هناك ارتباط بين السيرفايفين وبين شدة حب الشباب

كان هناك علاقة ايجابيه بين عامل النسخ وعامل النمو الشبيه بالانسولين -1,كذلك هناك علاقة ايجابيه بين عامل النمو الشبيه بالانسولين -1 والسيرفايفين وايضا هناك علاقة ايجابيه بين حيث تكون قيمة

$(p=<0.05)$

أظهر اختبار منحني روك قيمه تميزية جيدة للسيرفايفين وعامل النسخ بين مرضى حب الشباب والاصحاء

تشير النتائج التي توصلنا إليها إلى أنه عامل النمو الشبيه بالانسولين- 1و السيرفايفين وعامل النسخ يمكن أن يلعبوا أدوارًا محتملة في التسبب في حب الشباب.