عزل وتشخيص بكتريا

Propionibacterium acnes

من المرضى المصابين بحب الشباب

رسالة مقدمة الى مجلس كلية الطب – جامعة بابل كجزء من متطلبات

نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالبة

دينا محمد رؤوف الخفاف

٢٠٠٥

١٤٢٦

الخلاصة

من مجموع ١١٠ مسحة جلدية تم جمعها من المركز الأستشاري لمستشفى مرجان ومركز الحساسية التخصصي في الحلة ومن كلا الجنسين حيث كانت عند الاناث اكثر (٦٢.٧%) وبلغ انتشار حب الشباب اعلى معدل له (٥٨.٩) في الفئة العمرية (٢٠-١٦) سنة بينما يقل كلما تقدم العمر وخاصة بعد عمر ٣٠ حيث كانت هناك اصابة واحدة فقط.

تم عزل وتشخيص ثلاث عزلات من البكتريا اللاهوائية *Propionibactrium acnes* الموجبة لصبغة غرام والتي تعطي تحلل قليل او نادر من نوع β-hemolysis على اكار الدم المغذي حيث كانت عزلة واحدة فقط موجبة لإنتاج الهيمولايسين بينما كانت كل العزلات موجبة لإنتاج إنزيمي اللاييز والبروتيز , وحاوية على المحفظة وانتاج عامل الاستيطان الثالث (CFA/III)

تم اختبار حساسية هذه البكتريا لعدد من المضادات الحيوية المستعملة لحب الشباب وكانت متحسسة لكل من التتراسايكلين والدي اوكسي سايكلين والكلورامفينكول و النيومايسين والكفلككسين بينما أظهرت مقاومتها لكل من الأرثرومايسين والجنتامايسين.

وتم ايضا دراسة تأثير العلاج الموضعي لفيتامين A المسمى retinoic acid ولوحظ حساسية البكتريا له حيث يقل تركيز البكتريا عند التراكيز التي تفوق ٢٠ مايكروغرام/مل. كما ان للحرارة تأثير قاتل على هذه البكتريا خاصة عند الدرجات التي تفوق ٥٠°C .

أظهر فحص عينات دم بعض المصابين ارتفاعا ملحوظا في نسبة كل من lymphocytes بمعدل (٣٣%) وارتفاعا أكثر في monocytes بمعدل (١٢.١٥%) وكذلك ارتفاعا في الأستجابة المناعية المتخصصة بارتفاع تركيز IgG في الدم الى حوالي (١٥٣٠.٠٣٥ mg/dl) عند المصابين.

Isolation and characterization of *Propionibacterium acnes* from patients with Acne vulgaris

A Thesis

Submitted to the Department of Microbiology, College of Medicine, University of Babylon, in Partial fulfillments of the Requirements of the Master of Science Degree in Medical Microbiology

By

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We certify that this thesis was prepared under our supervision at the Collage of Medicine, University of Babylon, as partial requirements for the degree of Master of Science in Medical Microbiology.

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According to the available recommendations, I introduce this thesis to discussion.

Signature

Dr. Mohammed Sabry

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Abstract

In this study, ١١٠ skin swabs were obtained from patients suffering from acne vulgaris from consultation unit in Marjan Hospital, and Allergy specialized center in Hilla from both sexes .It was found that acne vulgaris was higher in female (٦٢.٧%) and more prevalence in (١٦-٢٠) years patients and lower in older especially after ٣٠ year age where there is only one case.

Three isolates of anaerobic gram positive *Propionibactrium acnes* were identified and diagnosis by culture and biochemical tests, all bacterial strains possessed capsule and produce lipase and protease enzyme and contained the third colonization factor antigen(CFA/III) while only one strain produce a narrow zone of β-hemolysis.

The effect of some antibiotics on *Propionibactrium acnes* was investigated, and the results showed that all isolates were sensitive to Tetracycline, Deoxycycline , Chloramphenecol, Neomycine and Kephalexin while they were resistante to Erythromycin , Fusidic acid , Vencomycon, Lincomycin and Gentamycin.

Also retinoic acid effect on bacterial growth was studied. It was stated that lactic acid at high concentration ٢٠μg/ml could cause inhibition to *Propionibactrium acnes* growth. Also temperature higher than ٥٠^{o}c would kill this bacteria.

Immunological response to *Propionibactrium acnes* was also recorded in both cellular and hummer response where IgG concentration significantly increase with blood sample of acne patients.

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Recommendation

- More studies about *Propionibacterium acnes* specially its chemotactic enzymes.
- More genetic studies to *Propionibacterium acnes*.
- Using both systemic and tropic treatments for *Propionibacterium acnes*.

بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العلي العظيم

(سورة البقرة الاية : ٣٢)

Dedication

To those who were patients with meMore than me,
My Parents…
My husband…

Dina

Acknowledgements

I am so grateful to the head of Microbiology Department in Medicine College Dr. Mohammed Sabry and to Dr. Mohemmed Aboud Al- Qaraguli .

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My thanks are to my friends in colleagues for their generosity and co-operation.

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Introduction

Acne vulgaris is a chronic inflammatory disease of the pliosebaceous unit (sebaceous gland and hair follicles) frequently occurring in younger population groups. In principle, it is a disorder of adolescence, but it persists unite the middle age in a minority of individuals. Acne vulgaris occurs in several clinical form. Severe forms of acne are often therapy- resistant which has mostly a negative impact on the patients.

The disorder of the pilosebaceous unit is of a multifactorial character. The proportion of individual pathogenic factors including genetic ones can differ with a resulting variability of clinical forms of acne. In many cases, the influence of various etiopathognic factors is combined (Yeon, et.al., ٢٠٠٠).

In patients, an increased production of sebum is manifested, and its quantity and quality vary depending on the hormone regulation. In general, androgens stimulate the formation of sebum, while estrogens on the other hand reveals a suppressive effect on it. The activity of sebaceous glands is thus dependent on the ratio of estrogens and androgens. The increased level of androgens in adolescence is known to be a starting point for the development of juvenile acne. The amount of triglycerides in sebum increases up to ٥٠٪ and together with their quantity, population of propionibacteria is enlarging, too (Webster, ١٩٩٩).

In acne, keratinization is also impaired. An increased production of keratinizing cells in follicular canals together with a decrease of their elimination leads to the accumulation of sebaceous matter. The primary manifestation of acne forms a comedo. In the formation of comedos, essential fatty acids are also involved (Federman, et.al., ٢٠٠٠) .

In the pathogenesis of acne, the natural skin microflora plays a marked role too, namely the gram-positive anaerobic rod *Propionibacterium acnes.* These bacteria present a number of enzymatic activities such as lipase, protease, phosphatase, neuraminidases and deoxyribonucleases.

Propionibacteria influence both cellular and humoral components of the immune system. They are able to persist in macrophages and increase chemotaxis of polymorphonuclear leukocytes.

They are also cytotoxically active, and able to activate the complement in alternative way and bring about hypersensitivity of early or late type. There are no sufficient studies concerning *Propionobacteria acnes* in Iraq, so this study aims to :

A. Isolate and characterize the bacteria associated with acne vulgaris (aerobic and anaerobic) particularly *Propionibacterium acnes* .

B. Study the effect of some antibiotics on *Propionobacteria* isolates.

C. Study of some factors associated with the pathogenecity of *Propionobacteria* such of adherence factors, lipase and protease.

D. Study some immunologic factors associated with *Propionobacteria invasion.*

١.٢ Literature Review

١.٢.١ Acne Vulgaris

Acne vulgaris is a chronic inflammation disease of the pilosebaceous unite, primarily of the face, nose, upper base, chest, shoulders and upper arms. It is one of the comments dermatoses affecting the teenage population (Sehgar & Jain, ١٩٩٥).

On the face, it occurs most frequently on the cheeks, and in lesser degree on the nose, forehead and chin (Andrew, ١٩٩٠).

Acne that start usually between the ages of ١٢ and ١٤ years, tending to be earlier in females. The peak age for severity in females in ١٦-١٧ and in males ١٧-١٩ years (Lucky, et.al.,١٩٩١).

Acne frequently occurs in individuals with high sebum production and bacteria is observed at acne regions. Products generated by ingestion and degradation of sebum by *Propionibacterium acnes* are considered to cause inflammation around pores, and aggravate the symptomes (Ingham,*et.al.*,١٩٩٢).

Endocrine, keratinization, and bacterial factors are considered to be the major factors in acne development (Aizawa & Niimura, ١٩٩١).Free fatty acids produced by *P.acnes* derived lipase, which induced impairment of keratinization and sebum accumlated in the hair follicular duct (Downing, *et.al.*,١٩٨٦).

When untreated, acne usually lasts for several years until it spontaneously remits (Strauss & Thiboutat, ١٩٩٩).

١.٢.٢ Pathogenesis

Adolescent acne (acne vulgaris) has a strong tendency to be hereditary and is less likely to be seen in Asian and dark skinned people (Herbert , ٢٠٠٠).

The pilosebaceous follicle is the target organ in acne, explaining the distribution of acne primarily on the face, chest and areas with the greatest concentration of pilosebaceous glands (Dreno & Khammari, ٢٠٠٠).

The most notable path physiologic factors that influence the development of acne are as follows :

١- Abnormal follicular keratinization . The earlist –change in the formation of acne occurs in the horny cells that line the sebaceous follicle. Adisturbance in the differentiation of these cells leads to excessive shedding of the cells into the human. Impaction of the follicle occurs as these horny cells stick together.

٢- Overproduction of sebum. The sebaceous gland is highly responsive to hormonal stimulation , namely androgens. With the rise of androgens, hypertrophy of the sebaceous gland occurs and production of sebum is increased.

٣- Proliferation of *P. acnes* . The over production of sebum in acne provides a good environment for proliferation of *P. acnes* (Jeffery, ١٩٨٢) . *P. acnes* possesses a lipase that hydrolyzes sebum triglycerides into free fatty acids. This initiates the follicular wall and cause inflammation.

٤- Inflammation. The expulsion of sebum into the dermal layer of the skin occurs as a result of the rupture of the pilosebaceous follicle. The initiates an inflammatory process and the formation of inflammatory lesions such as pustules and cysts .

Acne lesions are designated as inflammatory, comedonal (non inflammatory), or a combination of the two .

The non inflammatory lesions are represented by microcomedones and comedies. The microcomedon is the first pathogenic feature of acne that can be found in normal-looking skin and only be identified microscopically (Norris & Cunliffe , ١٩٨٨).

The fact that acne does not occure simultaneously on all susceptible sites is constant with the finding that sebum excretion varies from follicle to follicle (Pierard, ١٩٨٦) . This suggests that certain follicles may be prone to acne. An enhanced peripheral response must thus be considered as a factor in many subjects.

A comedo is a collection of sebum and keratin that forms within follicular ostoa (pores) and can be classified into either :

A. Open comedones (blackheads) have large ostia that are black not from dirt but from melanin .

B. Closed comedones (whiteheads) have small ostia (Herbert ,٢٠٠٠) .

Inflammatory acne lesions consist of papules , pastules , nodules and cysts . In these lesions , the follicular epithelium is

damaged , and a dermal inflammation occurs . Duct rupture can occur but is certainly not an early phenomenon (Cunliffe , ١٩٩٨) If rupture occurs the comedo content elicits an intense inflammatory respose in the dermis. Long-lasting dermal inflammation may give rise to induration nodules .

١.٢.٣ Epidemiology of Acne

Acne is the most frequent reason for consulting a dermatologist in private and, in recent years, has become not only an illness of teenagers, but also of adults . The mean onset of acne in girls is ١١ years and in boys ١٢ years. The mean prevalence is high – between ٧٠٪ and ٨٧٪ without any significant difference according to the country of origin of the patient (Dreno & Poli, ٢٠٠٣).

Among the prognostic factor of acne patients, with risk of no response to treatment or frequent relapses, the most important two that have been identified are genetic factor, with a history of acne in the family, and early onset of acne lesions before puberty. Moreover, recent studies seem to indicate that acne is more frequent in smokers (Schafer, *et. al*., ٢٠٠١).

An evaluation of the difference in acne according to skin colour has been preformed. The mean age of acne onset appears lower in Hispanic subjects (١٥ years old) compared with Black (٢٠ years old) and Asian (١٨ years old) subjects (Taylor, *et. al.*, ٢٠٠٢). Acne in black Americans is less evident than in white, who also have more sever acne than Japanese, that eating a diet

rich in fish, but increases markedly when they change to a Western , Canadian , diet with more saturated fats. (Hamilton, *et.al*., ١٩٦٤).

The frequency of acne lesions in adults has increased in the last ١٠ years. Acne in adult women appears particularly persistent and recurrent, including psychological disturbance, severe impact on quality of life with frequent sick leave taken as a result. The etiology of adult acne remains obscure, with cosmetic abuse and stress being suspected causes (Poli, *et. al.* , ٢٠٠٠).

١.٢.٤ Types of Acne

Differential diagnosis is important for the differentiation of acne caused by external factors and acne form.

١. Infantile acne: may follow transplacental stimulation of a child's sebaceous gland by maternal androgens.

٢. Mechanical : excessive scrubbing, picking , or the rubbing of chin straps or a fiddle can rupture occluded follicles. (Hunter, *et. al*., ٢٠٠٢).

٣. Acne associated with virilization : inducing clitoromegaly, may be caused by an androgen-secreting Jaws.

٤. Acne accompanying the pdycystic ovarian syndrome is caused by modestly raised circulating androgen levels. (Drake, ١٩٩٠).

٥. Drug – induced : prolonged application of topical steroids especially on the face , systemic corticosteroids, anderocorticosteroids, lithium , iodides, and bromides may result in acne . The lesions consist of small papules and pustules localized mainly on the trunk. (Sehgal & Jain, ١٩٩٥).

٦. Tropical: Heat and humidity are responsible for this variant, which affects Caucasoids with a tendency to acne.

٧. Acne cosmetica : Certain cosmetics induce acneiform eruption and are responsible for persistant low-grade close comedones on the face.

١.٢.٥ Etiology

Many factors may combine to cause acne, characterized by chronic inflammation around pilosebaceous follicles. There is undoubtedly a hereditary factor. Several members of the same family may be affected with severe scarring acne (Lucky, et.al., ١٩٩١) .

Basically, however, the primary defect in acne is the formation of a keratinous plug in the lower infundibulum of the hair follicle. Two major factors in the formation of these plugs are **:** androgentic stimulation of sebaceous glands (Walton , et.al. ,١٩٩٥) , and colonization of follicles by *Propionibacterium acnes* , which metabolizes sebum to produce free fatty acids . The

occurrence of androgen secretion at the onset of puberty explains the usual onset of acne at that age . Much evidence suggests that liberation of free fatty acids by the metabolic activity of *P. acnes* is a major factor in the genesis of acne papules and pastules (Andrews , ١٩٩٠) .

There are some factors which may lead to aggravate acne **:**

١- Dietary factors . There is no link between acne severity, caloric intake, carbohydrate, lipids proteins, minerals, amino acids or vitamins (Frish, ١٩٧٢).

٢- Changing climate . Acne increases in winter and cold weather (AL-Anbakey, ٢٠٠٠)

٣- Stress. Increase in cortisol lead to increase in sebum production , also acne itself induces stress, and 'picking' of the spots will aggravate the appearance (Bach and Bach , ١٩٩٣). Severe acne may be related to increased anger and anxiety (Kinder and Trunnen , ١٩٨٨).

٤- Sex hormones . Seboceous activity is predominantly on androgenic sex hormones of gonadal or adrenal origin . Abnormally high levels of sebum secretion could thus result from high over all androgen production , or increased availability of free androgen , because of a deficiency in sex-hormone-binding globulin.

٥. Cosmetic and skin care product and also, some drugs play a role in causation of acne.

١.٢.٦ Anatomy and Normal Flora of the Skin.

The skin is composed of two layers: the epidermis and dermis . The epidermis , the surface layer of the skin , ranges from ٠.٠٠٧ to ٠.١٢ mm thick . The outer portion is composed of scaly material made up of flat cells containing keratin . The cells on the skin surface are dead and continually peel off , and are replaced by cells from deeper in the epidermis (Kerr , *et ,al* .٢٠٠٣) .

The epidermis is supported by the dermis , a second deeper layer of skin cells through which many tiny nerves , blood vessels , and lymphatic vessels penetrate fine tubules of sweat glands and hair follicles traverse the dermis and epidermis . One or more pilosebaceous glands that produce an oily secretion called sebum , this secretion flows up through the follicles and spreads out over the skin surface , Keeping the hair and skin soft , pliable , and water repellent . (Nester , *et. al.* , ١٩٩٨)

The secretions of the sweat and sebaceous are very important to the microbial population of the skin because they supply water , amino acids , and lipids , which serve as nutrients for microbial growth.The normal PH of skin ranges from ٤.٠ to ٦.٨ (Marples , ١٩٧٤) .

Breakdown of the lipids by the microbial residents of normal skin results in fatty acid by products that inhibit the growth of many potential disease producers . In fact , the normal skin surface is a rather unfriendly terrain for most pathogens , being ,

unstable , acidic , and toxic for their survival (Nester , *et.al.,* ١٩٩٨) .

Large numbers of microorganisms live on and in the various components of the normal human skin. For example , the number of bacteria on the skin surface may range from ١,٠٠٠ organisms per square centimeter on the back to more than ١٠ million on the scalp and in the armpit (Nestor ,*et.al.,* ١٩٩٨) .

The normal floral includes yeasts and bacteria. The yeasts belong primarily to the genus Pityrosporum. *Staphylococcus* , *Micrococcus* , and *Corynebacterium* are commonly found on the skin (Kerr ,*et.al.,* ٢٠٠٣) . The prominent bacteria are *Staphylocooous epidermidis* ,and *S. aureus* , *Micrococcus luteus* , and *Propionibacterium acnes* (Todar ,١٩٩٧) .

١.٢.٧ *Propionibacterium* and acne vulgaris

This genus is so named because they produce propionic acid as principal product of their fermentation . They are gram-positive aerotolerat fermentative bacteria (Baron ,*et.al.,* ١٩٩٤) . They are found in two different habitats. One habitat occupied by the classic type of *Propionibacterium* is the intestinal tract of animals. The other major habitat of this bacteria is the skin of mammals. One species, *Propionibacterium acnes* , is found on the skin of all humans. It grows in the sebaceous gland (Perry & Staley , ١٩٩٧) .

There are three major subgroups of the propioni- bacterium *P.acnes* , *P.granulosum* and *P.avidum* . Almost centainly

P.acnes and , to a lesser extent , *P.granulosum* are the most important (Holland , *et.al.*, ١٩٧٨) .

Propionibacterium species are often referred to as the "anaerobic diphtherias" and belong to the genra of coryneforms because the bacilli are irregular, pleomorphic , and often clup shaped (Baron ,*et.al.*, ١٩٩٤).

These species are slow-growing , gram-positive , grows well at low oxygen tensions , catalase and indole positive (Gribbon , *et.al.*,١٩٩٤) .

Colonies of *P.acnes* on anaerobe blood agar are ١ to ٢ mm in diameter, circular, entire convex, glistening, and opague (Koneman ,*et.al.*,١٩٩٧)

Some strains produce a narrow zone of hemolysis. Propionibacterium species form propionate and acetate as the main metabolites from glucose . Smaller amounts of succinate , formate , lactate , and CO_2 may also be formed (Baron , *et.al.*,١٩٩٤) .

P.acnes produce several enzymes that contribut to pathogenesis such as lipases , proteases , neuraminidase , hyaluronidase and acylneuraminic acid (Rollins , ٢٠٠٠) .

P. acnes colonizes sebaceous follicles in the relatively anaerobic lipid-rich microcomedones . *P. acnes* produces an extracellular lipase that hydrolyze sebum triglycerides to glucerol , a growth substrate , and to free fatty acids , which may contribute to the formation of the comedone and induce inflammation . In

addition, *P. acnes* produces enzymes that lead to the rupture of the comedonal wall (Oberemok & Shalita , ٢٠٠٢) .

In some studies the number of *P. acnes* was found to be increased in acne lesions, but there was no correlation between the number of *p. acnes* and the severity and outcome of the disease . Other studies found no difference between the number of *p. acnes* in affected follicles and control ones (Leeming *et.al.,* ١٩٨٨) . Sebum excretion rate and ductal conification , by contrast, both correlate with clinical severity (Cove *et.al.*, ١٩٨٠). *P. acnes* live in skin in association with *S. epidermidis* and normal skin flora fungi, these organisms probably have some control over the growth of *P. acnes* (Holland , *et.al.*, ١٩٧٨).

The environment of this bacteria is probably more important than their absolute numbers for the development of acne lesions. It has been shown that oxygen tension, temperature and nutrient supply markedly affect the growth of P. acnes and the bacterial production of active substances such as lipases and proteases (Gribbon, *et.al.*, ١٩٩٤).

١.٢.٧.١ Virulence factors of Propionibacterium

The microbial factors that contribute to the virulence of a microorganism can be divided into three major categories :- Those that promote colonization of host surface, - those that evade the host's immune system and promote tissue invasion, and -those that produce enzymes and toxins that result in tissue

damage in the human host . Pathogenic microorganisms may have any or all of these factors (Murray , ٢٠٠٣) .

Propionibacterium acnes has gelatin layer capsule that avoid phagocytosis .This capsule may be weakly antigenic or strongly antigenic depending on their chemical complexity .This factor severe a diversity of functions in disease including : Antiphagocytosis and prevention of neutrophil killing of engulfed bacteria , prevention of complement-mediated bacterial cell lysis and protection of anaerobes from oxygen toxicity (Brooks *et.al*., ٢٠٠١).

P. acnes reside within the pilosebaceous unit in a biofilm . As such , they live in a community of bacteria that increase themselves with in an extracellular polysacchride lining , which the organism secrete after adherence to the surface (Perry and Staley ,١٩٩٧) . This glycocalyxpolymer acts as protective exoskeleton and serves as a physical barrier , limiting effective antimicrobial concentrations within the biofilm microenvironment (Baron *et.al*., ١٩٩٤).

The glycocalyxpolymer secreted by *P. acnes* as a biofilm may explian the immunogenicity of the organsim as well as the clinical course of the disease .

Besides, adhesion includes the interference between special receptor on surface of cell membrane in mammalian which represented by carbohydrate with receptors on bacterial surface which represented by proteins and for bacterial colonization the

adhesion is the first and the necessary step in bacterial infection of tissue (Hoeley *et.al.*, ١٩٩٧).

The adherence phenomenon is particularly important in mucosal surfaces which are washed continually by fluids. Bacteria that have pili and / or fimbriae can colonize that site .

P. acnes colonize hair follicle by possessing a number of enzymatic activity , this non-motile bacteria do not have any surface appendage on cell membrane (Murray ,٢٠٠٣).However, the first fimbrial factor is colonization factor antigen (CFA/I) that isolated from *E .coli* ,this factor causes (group A) RBC's human agglutination . Other factors named (CFA/II) that cause agglutination in cow and chicken blood. These two factors do not cause inhibition in presence of D-mannose (AL-Zaag,١٩٩٤) .

The third colonization fimbrial factor is (CFA/III) that cause RBC's agglutination in presence of Tannic acid (Hornic *et.al.*, ١٩٩٥) .

Once a microorganism adheres to a body site , it has an obligate requirement for iron for its subsegment growth and multiplication (Salyers and Whitt ,١٩٩٤) .

Microorganisms produce siderophores which chelate iron with a very high affinity and which compete effectively with transferrin and lactoferrin to mobilize iron for microbial use . In addition , some microbial species can utilize host iron complexes directly without the production of sidrophores . For example , *Yersinia pestis* can use heme as a sole source of iron ;

Vibrio can utilize iron from the hemoglubin-haptoglobin complex . Another mechanism for iron acquisition in the production of hemolysins ,*P.acnes* thought to be able to use this mechanism , which act to release iron complexes to intracellular heme and hemoglobin (Litwin and Colderwood ,١٩٩٣).

١.٢.٧.٢ Enzyme system

١-Lipase

Lipase is an enzyme that catalyzes hydrolysis of fatty acid ester bond in triacylglycerol (TAG) thus releasing free fatty acids. Note the reaction is reversible so the enzyme can catalyse esterification of glycerol to form mono-di and triglyceride (Shalita, et.al.,١٩٨٢) .

Lipase , the extracellular enzyme derived from P.acnes , is reported to be responsible for the hydrolysis of sebum triglycerides to free fatty acids which have been implicated as both irritants and comedogenic agents and which lead to an intensification of the inflammatory process (Freinkel , ١٩٦٩) .

P. acnes lipase and the majority of microbial lipases have been reported to be large molecules of aggregates of protein-lipid or protein-protein .

P. acnes lipase cleaves sebaceous diacylglycerol and triacylglycerol to glycerol and free fatty acids that induce proliferative hyperkeratosis in the

follicular canal , thus producing a comedogenic Effect. Its irritating effect(pH decrease) lead to the impairment up to the rupture of comedo covering (Biomed , ٢٠٠٢) .

There are studies shown that the chemotactic activity of *P. acnes* lipase act directly toward neutrophils in the absence of serum (Wilkinson, *et.al.,*١٩٧٣).Thus, it acts as stimulus of the reticuloendothelial system, as an adjuvant to enhance humeral and cell-mediated responses, and as antitumor agent which modifies the immunological response in tumors (Adlam & Scott ,١٩٧٣)

Most lipases have optimum temperature ranging from ٣٠-٤٠ c, but it should be recognized that most enzymes stop catalysis at –١٠ c because liquid substrates become solid ice . Lipases act on insoluble substrates ,and they have next to no activity against soluble substrates ,so the insoluble substrate must activate the enzyme (Arzoglou ,*et.al.,* ١٩٨٩) .

٢- Proteases .

Proteolytic enzyme or proteases catalyses the cleavage of peptide bonds in proteins. These enzymes are involved in essential biological processes like blood clotting, controlled cell death, and tissue differentiation. They catalyze important proteolytic steps in tumor invasion or in infection cycle of a number of pathogenic microorganisms .

Proteases of propionibacterium enable permeation of follicle content through its wall and by supporting of p. hyaluronidases ,

bacteria spreading in the dermis. The clinical consequences of these processes are papules , pustules , in duration and abscesses (Biomed , ٢٠٠٢) .

Other products of *P. acne* are phosphatases , heamolysin , neuraminidases , deoxyribonucleases and namely substances close to prostaglandins with marked importance in the formation of inflammatory manifestations of acne (Burkhart ,*et.al.,* ١٩٩٩)**.**

١.٢.٨ Growth temperature .

Microbes are divided into four groups based on the range of temperature at which the can grow *P. acnes* can grow between ٢٠ C˚ and ٤٤ C˚ .

Sebum is a liquid material at body temperature but this characteristic is affected according to environment temperature . At ٢٥ C˚ sebum becomes semisolid and that effect on the viscosity and flow of the sebum . So the theory that assumes that the cold weather causes block ducts by solidification the sebum is right and acne increase in winter (Rodan & Fields ,١٩٩٦) .

١.٢.٩ Acne treatments

Depending upon the degree of involvement , treatment varies from topical application of mild lotions to systemic therapy with antibiotics or retinoids (Andrews , ١٩٩٠) . Acne treatments need extreme patience , as often no benefits are seen for six to eight weeks after starting therapy . It is important to keep up

with the treatment , however , as after two months of continued and regular use (Loutit, *et.al.*, ١٩٩٥).

The following measures are used progressively and selectively as the disease is more severe .

١.٢.٩.١ Abrasive agents .

Agents such as alumium oxide , polyethylene granules in detergent or soaps help to remove excess sebum and comedones and reduce the number of bacterial colonies on the skin . They are suitable for use in mild acne (Laurence , *et.al.*, ١٩٩٨) .

١.٢.٩.٢ Mild keratolytic .

Formulations unblock pilosebaceous ducts , e.g. benzoyl peroxide , sulphur , salicylic acid , and azelaic acid . Benzoyl peroxide . This antibacterial agent is applied as a cream , lotion or gel only at night initially , and has an antibacterial effect propionibacterium , and reduces the number and size of comedones and damps down inflammation , so that the number and size of inflammatory nodules is reduced (Hunter , *et.al.*, ٢٠٠٢) .

١.٢.٩.٣ Hormonal agents .

Elevated androgen levels promote acne , and agents that lower androgens are used to treat acne in females (Shaw & White , ٢٠٠٢) .

An oral contraceptive containing ethinyl estradiol and levonorgestrel was found to decrease inflammatory and

noninflammatory lesions and decrease in the serum levels of several free androgens in treated patients (Leyden , *et.al.*, ٢٠٠٢).

١.٢.٩.٤ Systemic or topical antimicrobial therapy .

Four oral antibiotic treatments are available **:** tetracycline ,doxycycline ,minocycline and erthromycin. Systemic antibiotics are useful for mild to moderate acne that affects a large area such as the face ,back and chest (Loutit, *et.al.*, ١٩٩٥) .

A. Tetracycline .

The usual dosage of tetracycline is ٢٥٠ mg three or four times daily (Laurance ,*et.al.*, ١٩٩٨). Tetracycline acts by killing *P. acnes* , by interfering with protein synthesis by binding to bacterial ribosome , and thus reducing the production of lipases in the sebaceous follicles (Andrews , ١٩٩٠) . In addition to direct antibacterial effects ,previous studies have suggested that antibiotics are beneficial in treating acne through their anti-inflammatory effects and inhibit the production of inflammatory mediators produced by pathogenic bacteria (Higaki ,*et.al.*, ٢٠٠٢) .

This antibiotic is bacteriostatic ,especially in large doses .In smaller doses oral antibiotics do not reduce the number of organisms but they affect their function. The antibiotics can also inhibit various enzyme activities (Webster ,*et.al.*, ١٩٨٢) and modulate chemotaxic ,lymphocyte function and pro-inflammatory cytokines (Eady ,*et.al.*, ١٩٩٣) .

B. Minocycline .

This antibiotic is much more lipophilic than other antibiotics and so probably concentrates better in the sebaceous glands .It is bacteriologically more effective than tetracycline and little resistant to it by propionibacteria has been recorded, but can cause abnormalities of liver function (Hunter ,*et.al.*, ٢٠٠٢) .

This antibiotic has shown that the absorption of it is reduced by food .It is very extensively used but it should not be the first-line in treatment of acne (Meyer ,١٩٩٦) .Resistance to minocycline is rare .

C. Deoxycycline .

It is used as alternative to minocycline ,but more frequently associated with phototoxic skin reactions (Hunter ,*et.al.*, ٢٠٠٢) .Doxycycline and minocycline are equally effective .*P. acnes* is not resistant to dexycycline (Olafsson ,*et.al.*, ١٩٨٩) .

D. Erythromycin .

Patients with severe acne should receive ٢٥٠mg orally four times daily for ٦-٨ weeks (WHO, ١٩٩٧). Its major drawback is the development of resistant propionibacterium which leads to therapeutic failure .

١.٢.٩.٥ Retinoid :

Is vitamin A (retinol) analogues .

Retinoid and its derivative remain the most effective treatment for acne but is reserved for severe cases because of the risk of side effects and the need for close monitoring during treatment (Celi, et.al., ٢٠٠٢) .

Retinoid ,like glycorticoids and thyroid hormone ,bind to cytoplasmic receptors including signals which in the presence of a ligand ,are transmitted to the nucleus (Craig, ٢٠٠٣). Retniods down-regulate lipid synthesis ,decrease late-stage sebocyte differentiation ,and inhibit sebocyte proliferation .Thus far ,the most potent retinoid in inhibiting proliferation and differentiation is isotretinoin (Kealey & Guy ,١٩٩٧) .

Topical retinoids target the microcomedo , which is the precursor of all other acne leasions .Topical retinoids reverse the abnormal dequamation by affecting follicular epithelial turnover and maturation of cell .Thus , retinoids are recommended at the initiation of therapy .In addition ,some topical retinoids have an effect on inflammation by modulating the immune response ,inflammatory mediators and the migration of inflammatory cells(Millikan ,٢٠٠٣) .

Combind with antibiotic therapy ,it provides greater and faster results compared with antimicrobials alone .

١.٢.١٠ Immunity with acne

The skin represents the largest organ of the human body. In addition to its structural functions , a specific immunological environment has developed in the skin . The professional immune cells are macrophages , neutrophils , dendritic cells and lymphocytes , and non-professional immune cells , such as keratinocytes and sebocytes (Zonneveld , *et.al.*, ١٩٨٧) .

The most important function of the innate immune system is to recognize foreign invaders self-molecules with modified structures . Abnormal reactions to such stimuli could lead to the development of pathological features . Considering the peculiar aspects of the pilosebaceous unit , which is constantly hosting *P. acnes* and is especially rich in lipids , two mechanisms could have an outstanding role in the development of acne lesions **:** recognition of pathogens by Toll-like receptors (TLRs) and presentation of abnormal lipids by CD١d molecules , which results in the activation of natural killer T cells. Natural killer T cells could further activate both the cells of the innate immune system and the cells of the adaptive immune system ; they represent a link between innate and adaptive immunity (Koreck ,*et. al.*, ٢٠٠٣) .

Several studies have demonstrated that in acne patients and especially in subgroups with severe forms , the immune response against *P. acnes* is abnormal and reported that patients with severe acne have significantly responde to *P. acnes* ,and

also showed that lymphocyte stimulation by *P. acnes* significantly increased in patients with nodulocystic acne (Puhvel ,*et.al.,* ١٩٧٧) .

P. acnes could activate both peripheral blood mononuclear cells of adults and cord blood monobuclear cells and that reactions produced are both antigenic and T cells mitogenic (Jappe ,*et.al.,* ٢٠٠٢) .

Recent investigations on the recognition of *P. acnes* have shown that this pathogen induces expression of IL-٨ in a TLR٢- and NF-kB. Dependent manner. CD١٤ is also involved in the *P. acnes* –induced up regulation of IL-٨ expression (Chen ,*et.al.,* ٢٠٠٢) .

Many strains of propionibacterium enhance cellular and humeral immunity in animals and in human and are sometimes used as adjuvants. Adjuvant activity is strain-variable and correlates with the ability to persist within macrophages (Baron,*et.al.,* ١٩٩٤) .

Because propionibacterium are potent adjuvant , their presence on human skin may constitute a first line immune defense system against microbial infection and cancer .

Material & Methods

٢.١ Materials

٢.١.١ Instruments

Table (١) shows laboratory instruments used in the work

Instruments	Company
Sensitive electronic balance	A & D, Japan
Autoclave	Stermite, Japan
Incubator	Memmert, Germany
Centrifuge	Hermle, Japan
Oven	Memmert, Germany
Refrigerator	Concord, Italy
Light microscope	Olympus, japan
Micropipette	Oxford, USA
pH meter	Hoeleze & Cheluis, KG,Germany
Inoculating loop	Japan
Inoculating needle	Japan
Spectrophotometer	Bausch & Lomb

٢.١.٢Chemicals

Table (٢) show chemicals materials

Chemicals	***Company***
NaCl, $MgSO_4$, Na_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, $FeCl_3$, KOH, K_2HPO_4	Merk-Darmstadt.
α-naphthol amine , tetramethyl-P-paraphyenylene diamine dihydrochloride, sulfunic acid, Acetic acid, Tanic acid, Olic acid	B.D.H.
D-Mannose, Glucose, Dipyridyl	Fluka chemika-Switzerland

٢.١.٣Culture media :

The following media is supplied from companies as listed below.

Media	**Source**
Blood agar base	Mast
Brain heart infusion agar	Mast
MacConkey's agar media	Mast
Mullar – Henton infusion agar	Mast
Nutrient agar media	Oxiod
Nutrient broth	Oxiod
Thioglycolate agar	Oxiod
Thioglycolate broth	Oxiod
Tryptic soy agar	Diffco
Pepton water media	Mast

٢.٢ Patients and Methods

٢.٢.١ Patients

١١٠ samples were collected from acne patients; their ages range from ١٣-٣٠ years old. The patients were suffering from sever to moderate inflammatory acne, with papule. Also the history of disease obtained from each patient was according to the following formula:

Name.

Age

Sex

Using

Using of Antibiotics

Duration

Inheritance state

٢.٢.٢ Specimens collection

Ethyl alcohol ٩٩٪ is used to disinfect the skin area before obtaining the swab. The technique of sampling is very important & containing the pus present . The obtained material is inoculated on appropriate culture media for anaerobic bacteria (thioglycolate agar) and on blood agar by using gas pack gar, for ٤٨ hr. in ٣٧ C^{o} .

٢.٢.٣. Synthetic media

١. *M ٩ agar*

The composition of this media is as the following

٦ gm of Na_2HPO_4.

٣ gm of $KHPO_4$.

٠.٥ gm of NaCl.

١ gm of Ammonium chloride.

Dissolve all these in ٩٥٠ ml of distal water and sterilized in autoclave then add ٢ ml of ١ M magnesium sulfate, ١٠ ml of ٢٠٪ glucose , ٠.١ ml of ١M $CaCl_2$ are added then complete the volume to ١٠٠٠ ml (Miniatis, et.al., ١٩٨٢).

٢. *lipolytic agar*

The media contains the following ingredients ;

٠.١ gm of Peptone.

٠.٢٥ gm of sodium chloride.

٠.٠٠٥ gm of Calcium Chloride.

٢ gm agar.

All these are suspended in ٥٠ml of distal water, containing ٠.٥ ml of Oleic acid.

٣. *Esculin agar*

This prepared according to (Diffco).

The ingredients of this media are as follows :

٤٠ gm heart infusion agar.

١ gm Aescoline.

٠.٥ gm Ferric Chloride.

All ingredients are dissolved into ١ L of distal water and dispense in to test.

٢.٢.٤ Reagents and solutions

١. *Kovac's reagent.*

It prepared by dissolving ٥ gm of (Dimthylamin Benzylaldehyde) in ٧٥ ml Amyl Alcohol, then ٣٥ ml of HCl is added . This reagent is used for detection of Indole (Macfaddin, ١٩٧٩).

٢. *Nitrate reduction reagent*

This is prepared according to (Collee, et.al.,١٩٩٦)

First solution is prepared by dissolving ٨ gm of sulfunilic acid in ١٠٠٠ ml of ٥ M acetic acid .

Second solution is prepared by dissolving ٥ gm of alpha-naphthyl amine in ١٠٠٠ ml of ٥ M acetic acid.

Equal volume of each solution is mixed to prepare the reagent.

٣. *Catalase reagent*

is prepared by adding ٣ gm of H_2O_2 to ١٠٠ ml of distill water and stored it in dark container.

٤. *Phosphate Buffer solution*

It contains:-

٨٠ gm of Sodium chloride

٠.٣٤ gm KH_2PO_4.

١.١٢ gm KHPO٤ .

All these ingredients are dissolved in ١ L of distal water then the pH of solution is adjusted to pH=٧.٣

٥. *Deoxycycllin solution*

٠.٢ gm of deoxycycllin powder is dissolved in ٩٠ ml of ٥٠٪ ethanol then the volume is completed to ١٠٠ ml (the concentration is ٢٠µg/ml)

٦. *Tetracycline solution*

Dissolve ٠.٢ gm of antibiotic in ٩٠ ml of ethanol alcohol ٩٩٪ then complete the volume to ١٠٠ ml to get ٢ mg\ml as final concentration (Miniatis, et,al., ١٩٨٢).

٢.٢.٥ Stains

١. Gram stain :

This stain is used to differentiate gram negative and gram positive bacteria

٢. Albert stain:

This stain is used to diagnose *Corynbacterium spp*.

٣.Leishman stain:

This stain is used to stain blood film.

٢.٢.٦ Isolation & identification of *Propionibacterium acnes*

From the inoculated blood agar and thioglycolate agar that incubated aerobically for ٢٤ hr. Rod bacteria gram

positive by gram stain slide and Albert stain for further determination. The isolated strain is inoculated on Aesculine agar to show Aesculine hydrolysis .

٢.٢.٧ Biochemical test

- *Catalase test:-*

  Transfer a colony of the organism to be identified to a drop of ٣٪ H_2O_2 on a microscope slide. If Catalase is present, the formation of gas bubbles have occurred which indicates the positive results.

- *Indole production:-*

  A ١٪ solution of tryptone broth is prepared in the tubes then sterile it in the autoclave. After that inoculate the broth with bacterial isolate and incubate it for ٤٨-٧٢hr. at ٣٧C^O then add ٦-٨ drops of Kovac's reagent (P-dimethylaminobenzaldehyde in amyl alcohol) to test indole production. Ring at top of broth in red color is a positive reaction.

- *Nitrate reduction*

- *Escolin hydrolysis*

  The bacteria are grown in an esculin slant. The dark brown color is the positive results. The unchanging of the color is a negative reaction (Barid, ١٩٩٦).

٢.٢.٨ Detection of some factors

٢.٢.٨.١ Capsule

Capsule staining reagent composed of

١ gm of crystal violet.

٢٠ gm of copper sulfate.

١٠٠ ml of distil water.

Prepare a smear and don't fix, when dry, floud gently with the crystal violet and leave for about ٤ min. wash off with the copper sulfate, allow to dry in air.

The bacteria should be a deep purple, the capsule, blue against a light purple back ground. (Cruickshank, et.al., ١٩٧٥).

٢.٢.٨.٢ hemagglutenation test (HA)

Prepare ٣٪ RBCs (A,B or O)(٣ ml RBCs + ٩٧ ml D.W.). then prepare brain haert agar supplied with blood, inculate and incubate for ٢٤h.

٢٥ µm of RBCs +loopful from culture, mixed on slide and wait for ٥min (Diffco, et.al., ١٩٧٥).

٢.٢.٨.٣Production of hemolysin

Hemolysis production was shown on blood agar media. The bacterial isolate was inoculated un aerobically on blood agar plats and was incubated for ٢٤-٤٨hr. at ٣٧C^{o} and was detected if there was any Hemolysis presence around the colonies (De Boy, et.al., ١٩٨٠)

٢.٢.٨.٤Siderophore production

M٩ agar is used for sidrophore production which is supplemented by ٠.٢٥ gm glucose after sterilization, ٢٠٠ µm ,dipyridyl is added (Nassif, et.al., ١٩٨٩).

٢.٢.٨.٥Colonization factor antigen

١. ***CFA/ ١***

Inculcate bacterial strain on tryptic soy agar which is incubated in ٣٧ Co for ٢٤h.

-RBCs suspension was prepared from group A washing it with phosphate buffer saline (repeated ٣ times) then prepare ٣٪ suspension from RBCs (v\v).

bacterial suspension from that grown on TSA was prepared , and mixed with ٠.١٥M NaCl to determine RBCs agglutination test and was castigated on colonies factor antigen type ١.

On clean slide mixed on drop of bacterial suspension with one drop of RBCs suspention and one drop of ٠.١٥M NaCl (with out D-Manose).

Examine the glutination of RBCs on slid in room temperature and from ١\٢-٢ min. (Symth, ١٩٨٢).

٢. ***CFA\ II***

To determine second colonization factor antigen follow same step with (CFA\١), chicken blood used instead of group A blood.

٣. ***CFA \ III***

-Bacterial strain was inculcated on TSA and was incubated in ٣٧ Co for ٢٤h.

-RBCs suspension (A group) was washed with ٠.١M phosphate buffer solution.

-after growing of TSA mix with ٠.١٥M of NaCl.

-one clean slides RBCs suspension mixed with one drope of ٠.١M of Tannic acid in ٠.١٥M NaCl and one other slide mixed one drope of bacterial suspension with drop of NaCl ٠.١٥M without Tannic acid then the agglutination is shown in room temperature and for ١\٢-٢ min. (Symth, ١٩٨٢).

٢.٢.٨.٦Lipase production

Lipase production is detected according to the (Microbiological culture media, ٢٠٠٠) methods.

After preparation of lipolytic agar inoculation it with isolates and incubate it in ٣٧ C^o for ٢٤h.

Positive lypolysis is the presence of halo of precipitate surrounding the colony (Symth & Alford, ١٩٨٤).

٢.٢.٨.٧ Protease production

Protease was prepared by Piret, et.al., ١٩٨٣ method by using M٩ agar with glucose ٢٪ and gelatin ١٪

- inoculate this agar with bacterial isolation by picking and patching methd.
- After inocubation period ٢٤-٤٨h. in ٣٧ C^o add ٣ ml of trichloroacetic acid ٥٪ prespetate unlysis protein and view the transparence area around every colony to insure proteases production.

٢.٢.٩ Effect of incubation temperature on bacterial growth.

The isolation bacteria were re-inoculcated on nutrient agar and then incubated at various temperature degrees to show the effect.

٢.٢.١٠ Effect of Retinioc acid on bacterial growth

a. Nutrient broth is prepared and distributed in tubes and the Retinioc acid is added to each tube at various volume to gain the final concentration (١٠, ٢٠, ٤٠, ٨٠, ١٠٠ mg\ml).

b. Positive control is prepared by using Nutrient broth free from Retinioc acid.

c. The tubes in item ١ and ٢ are inoculcated with ٠.٥ ml of bacterial suspension and then inocubated for ٢٤h. at ٣٧ C^o.

d. After incubation, the obsorbtion is read at wave length ٥٢٠ nm.

e. Draw standard curve .

٢.٢.١١ Antibiotic sensitivity

١. By using (Disc method) Paur-Kirby method Muller-Hinton agar is prepared to use it with antibiotic discs (Al-Karky, ١٩٩٥) .

Isolates were inoculated then disc was put it on agar surface then was inocubated for ٢٤h. at ٣٧ C^o.

This procedure repeated twice, first after added human blood serum to the agar and the second by the addition of blood plasma to the agar.

٢. Muller-Hinton agar is prepared and sterilized in autoclave then added to it the antibiotic solution that

prepared previously then it was poured in sterile Petri dishes and the bacterial isolates were inoculated on media by Pinking and Patching method for ٢٤-hr. at ٣٧ C^o. the detection of sensitivity to antibiotic was done by presence of bacterial growth or not (Miniatis, et.al., ١٩٨٢) .

٢.٢.١٢ Immunity to acne

٢.٢.١٢.١ Differential Leukocyte Count (DLC):

Blood film was made immediately as follows:-

After careful mixing of the blood, an appropriate drop was delivered by a capillary glass and was placed in the center line of a clean microscope slide about one cm from one end. Then without delay, a spreader was placed in front of the drop at an angle of about ٣٠ْ to the slide and was moved back to make contact with the drop. The drop was spread out quickly along the line of contact. With a steady movement of the hand, the drop of blood was spread along the slide. The spreader was not lifted off until the last trace of blood has been spread out (Lewis et. al., ٢٠٠١). After air drying, the blood film was stained by Leishaman's stain (٣-٤-٢):-

The slide was flooded with the Leishman's stain. After two minutes, double the volume of buffered water was added for ٥-٧ minutes. Then washed in a stream of buffered water until it acquired a pinkish tinge (up to two minutes), the back of the

slide was wiped and set it upright to dry. Preparations were examined under oil immersion lens (١٠٠ X), counted ١٠٠ leukocytes and calculated the percentage of each type of leukocyte (Lewis et. al., ٢٠٠١,a).

٢.٢.١٢.٢ Radial immunodiffusion plates (IgG, IgM); produced by "**Biomaghreb**",

- Open the plate and let it stay for about ٥ min. at room temperature, allowing any possible condensation to evaporate.
- Fill well (or wells) with ٥ μl of serum or control sample using suitable device.
- Put a wet cotton or wet goze in the plate center to avoid agarose dehydration. Close plate tightly.
- Allow plate to stay flat at room temperature the time stated in reference table.
- Measure the diameter accurately to with in ٠.١ mm with a stable device.
- Evaluate resulte using the table of reference or standard curve.

Result and discussion

Acne is a common inflammatory skin disease due to infection of blocked hair follicles. Each hair follicle is associated with a sebaceous gland which secretes a conditioning oil (sebum) into the follicle. The condition usually starts in adolescence in both male and female.

٣.١ Acne distribution

In this study, ١١٠ patients suffering from acne admitted to unit of dermatology in Merjan hospital were included. It was seen that the distribution of acne vulgaris was higher among female than male (٦٢.٧٪ and ٣٧.٢٪) respectively.

This result was correlated with those results obtained by Kligman, ١٩٧٤ who had pointed that acne prevalence was more among females than in male, but it is not known why acne resolves or why it is more persistent in females, while Burton , ١٩٨٨ pointed that both sexes are equally affected. Also Burton et.al., ١٩٧١ said that acne was developed earlier in females than in males. Furthermore, the distribution of this disease according to the patient's ages was also studied. Most infection of acne vulgaris were showed at high rate (٥٨.٢ %) among the ages ranging from (١٦-٢٠) years old in both sexes. Burton results were identical with the results obtained by this study about the prevalence of acne in the ages between ١٥-١٧ years in females, and ١٦-٢٠ years in males.

Acne problem appear in older patients in mid-twenties (٢١%), Rea *et.al*., (١٩٩٧) stated that (١٨٪) of acne cases had late onset in the ages above ٢٠ years old.

Also this study established that (٤٠٥٪) acne patients had acne infection at the age of (٢٠-٣٥) years, this result was close to Stern, (١٩٩٢) and Fellowes *et.al*.,(١٩٨١) results which have ٥% and ١% of patients with acne in the same age groups. All these results and others are listed in table (٤).

Table (٤)

Distribution of acne infection among age and sex.

Age	*Sex*		*Number*	*%*
	Male	*Female*		
١٠-١٥	١	١٧	١٨	١٦.٣
١٦-٢٠	٣٣	٣١	٦٤	٥٨.٢
٢١-٢٥	٧	١٦	٢٣	٢٠.٩
٢٦-٣٠	–	٤	٤	٣.٦
٣٠>	–	–	١	٠.٩
Total	٤١	٦٩	١١٠	١٠٠
%	٣٧.٢	٦٢.٧		

Acne problem in adolescence may be due to excrete high quantity of sebum than normal people. Ebling (١٩٧٤) and Pochi *et.al*., (١٩٦٥) mention that sebaceous activity is predominantly

dependent on androgenic sex hormones. Furthermore there is general agreement that plasma testosterone levels are not abnormally high in males with acne (Pochi *et.al*., ١٩٦٥). However, some investigators have found raised levels of testosterone in acne patients(Carmina & Lobo, ١٩٩٣).

Burton, (١٩٧١) had suggested that other hormones affect the sebaceous glands, either directly or by enhancing their response to androgens, specially growth hormone.

Also, other studies have shown that genetic factors influence susceptibility to acne (Goulden *et.al*., ١٩٩٧ ;and Walton *et.al*.,١٩٩٨) and they have pointed that patients persistent acne have a strong family history of persistent acne in contrast with patients with adolescent acne.

Acne in adult women appears particularly persistent and recurrent, including psychological disturbance, severe impact on quality of life with frequent sick leave take as a result. Etiology of adult acne remains obscure, with cosmetic abuse and stress being suspected causes.

٣.٢ Isolation of bacteria from acne lesions

A total of ١١٠ samples were taken from acne lesions of acne patients, ٩٢(٨٣%) samples gave positive culture and only ١٨(١٦٪) samples gave negative culture. However, coagulase negative *staphylococcus* isolates were the predominant (٨١.٥٪), followed

by coagulase positive *staphylococcus* (٨.٦٪) , *Proipionibacterium acnes* (٣.٢٥l%) , Acinetobacter (٢.١٪) and the others belong to the yeasts table (٥)

Table (٥)

Frequency of Positive culture and negative culture

Number	*Positive culture*	*Negative culture*
١١٠	٩٢	١٨
%	٨٣. ٦%	١٦. ٤%

Table (٦) shows the number and percentage of bacterial species isolated from acne lesions. The first three bacterial isolates in this study are correlated with the three major organisms isolated from the surface of skin and duct of acne patients in previous studies where Marples, ١٩٧٤ and Nester *et.al*., ١٩٩٨ pointed that *Staphylococcus aureus, Staphylococcus epidermides* and *Propionibacterium acnes.*

The large group of microorganism universally present on the normal skin is the gram-positive cocci, staphylococcus.

Staph. aureus appear in low peresentage in this study (٨.٦٪), this S.aureus is the most pathogenic of the staphylococci.

The principal species in skin flora is *Staphylococcus epidermidis* which have high isolated peresentage (٨١.٥٪) in this study. It is also the percentage isolation in Marples and Nester studies.

S. epidermidis was found on surface of skin as well as inside duct of skin that explain the high isolation rate also Nester et.al.,١٩٩٨ showed that the main importance of skin's staphylococcus is probably in preventing colonization by other pathogens and in maintaining the balance among the skin's varios flora. These gram-positive cocci have been found to produce antimicrobial substances highly active against *P.acnes* and other gram-positive bacteria, while Perry and Staley, ١٩٩٧ referred that *P.acnes* ferments the lactic acid produced by S.epidermidis to from propionic and acetic acid.

All these factors and also, the living of *P. acnes* inside hair follical lead to hard difficulty of isolation of *P. acnes* from skin that lead to isolate only three isolates (٣.٢١٪) in this study.

Two isolates of Acinetobacter which were gram-negative had been isolated . These bacteria were widely distributed in population and widely distributed in soil and water that can occasionally be cultured from skin (Jawets, ٢٠٠١).

Tiny yeast almost universally inhabits the normal human skin. Holland *et.al*.,(١٩٧٨) said that *P.acnes* as they live in association with the *S. epidermidis* and fungi species, thus they later probably have some control over the growth of *P. acnes*.

Table (٦)

The number and percentage of all genra and species of isolates.

Type of bacteria	Number	%
Staphylococcus epidermidis Coagulase negative	٧٥	٨١.٥
Staphlococcus aureus Coagulase positive	٨	٨.٧
Propionibacterium acnes	٣	٣.٣
Acinetobacter	٢	٢.٢
Fungi	٤	٤.٣
Total	٩٢	١٠٠

Propionibacterium acnes on the other hand were considered as normal flora in the skin , however their role in acne is still vague. So this study may concern on this bacteria because there is no sulfured studies preformed on this bacteria , also there is no research in Iraq which covered the importance and pathogenesis of this through it's ability to produce certain virulence factor .

٣.٣ *Propionibacterium acnes*

Only three isolates of *Propionibacterium acnes* were cultured from the lesion of patients with acne vulgaris. All isolates were obtained directly from the pus formed in the bottom part of the inflamed follicle. In Iraq, this bacteria was isolated from patients with acne vulgaris particularly in Baghdad (Sabri *et.al*., ١٩٩٩ and Al-Anbaky, ٢٠٠٠) .

The most important characteristics of *P. acnes* colonies on blood agar are circular enter convex, white or yellowish colonies with weak or no hemolysin.

Direct microscopic examination showed that the bacteria is gram-positive irregular bacilli, highly polymorphic, showing curved clubbed, or pointed ends.

Koneman *et.al*., (١٩٩٧) and Perry and Staley,(١٩٩٧) suggest that this non – sporeforming bacilli typically slowly grow on obligate, however, some strains aerotolerant, but better growth was seen when the bacteria were cultured an aerobically, also they observed that these organisms u take the lactic acid formed by the lactic acid bacteria fermentation and further metabolizes it to propionic and acetic acid as well as CO_2

All isolates were grown an anaerobically on blood agar and on thioglycolate media. This bacteria can hydrolyze esculin, Catalase and Indol positive, all these tests and others listed in

table (٧) which are also listed in Holt *et.al*., (١٩٩٤) and Colle *et.al*., (١٩٩٦).

Table (٧)

Biochemical test used in identification of *Propionibacterium acnes*.

Test	Isoletes
Growth on thioglycolate	+
Growth on Blood agar	White to yellowish
Gram stain	+
Albert stain	+
Shape of cells	Bacilli, Polymorphic
Catalase	+
Oxidase	-
Escoline hydrolysis	+
Indol production	+
Nitrate reduction	+
Glucose	+
Hemolysis	Beta (Weak)

Negative staining technique was used to investigate encapsulated Propionibacterium capsulation and the results were positive for all isolates. This glycalyx polymer acts as protective layer, physical barrier encased in an extra cellular polysaccharide that they secrete after adherence to the pillar lining. This concept would clarify the unreliable correlation of antibiotic resistance with treatment outcome.

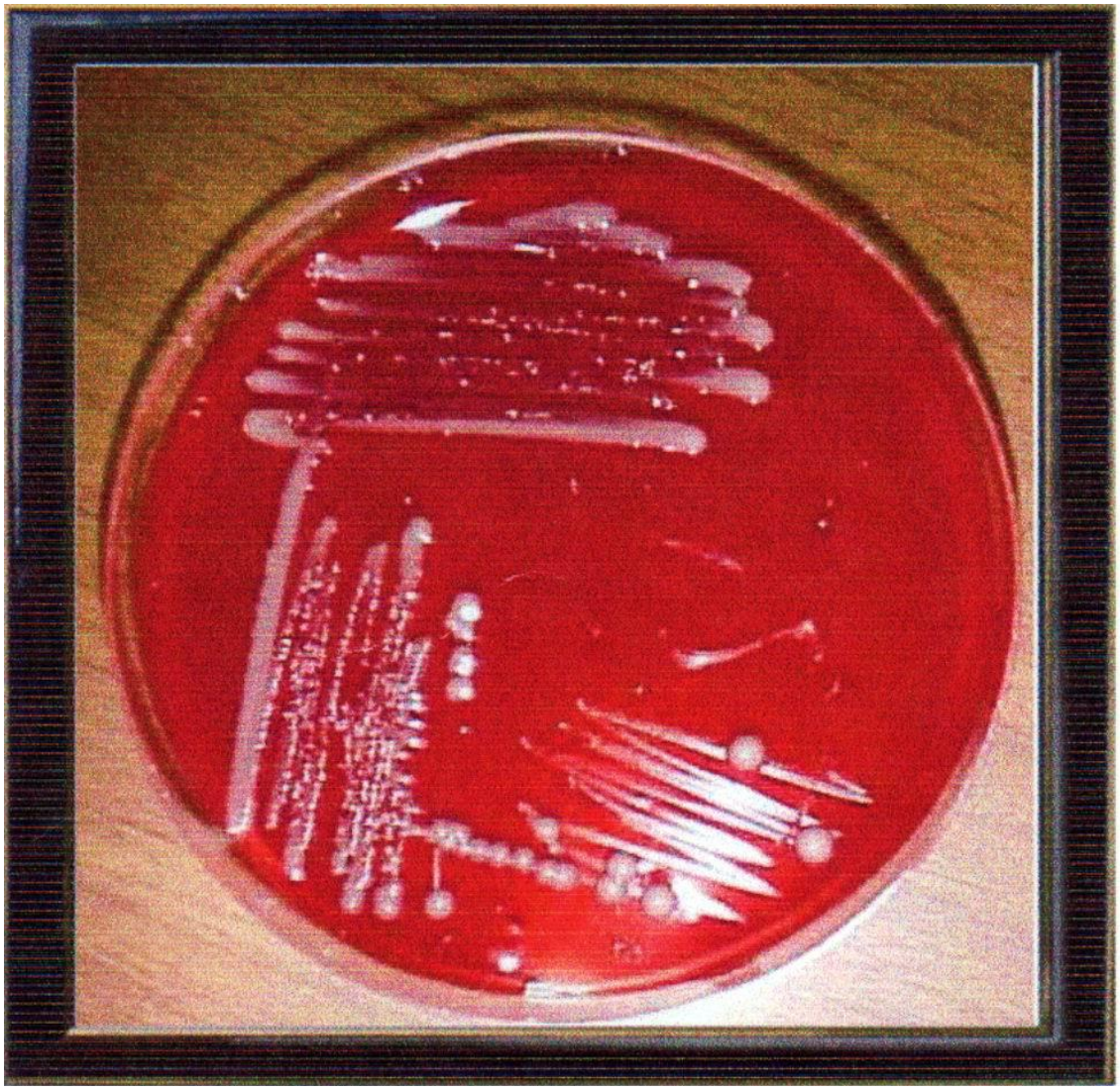


Figure (١)

Propionibacterium acnes **appearance on blood agar**

٣.٤ Virulance factors of *P. acnes*

Also table (٨) shows that only one strain was found to produce hemolysin that contribute with Koneman *et.al*., ١٩٩٧ results that reported only some strains produce a narrow zone of hemolysis.

Table (٨)

Detection of virulence factors among *Propionibacterium acnes* isolates.

Test	Isolate No.١	Isolate No.٢	Isolate No.٣
Capsule staining	+	+	+
Hemolysin production	+	–	–
Sidrophore production	–	–	–
CFA/I	–	–	–
CFA/II	–	–	–
CFA/III	+	+	+
Hemagglutuenation test	–	–	–

The isolates of *P. acnes* were tested for their possessing colonization factor antigens, and the results showed that all isolates did not have the activity to produce CFA/١ andCFA/٢.

In contrast, all isolates gave positive-results in agglutination test with (group A) human RBC's in the presence of Tannic acid which might refer to the fact that all isolates had colonization factor antigen type III(CFA/III). These results give clear evidence that all strains of *P. acnes* have one type of colonization factor that give high activity in adhesion that increase infection in human body (Neesif *et.al*., ١٩٨٩).

Also, there is strong evidence that CFA are important in evoking resistance or immunity, against disease caused by this pathogen (Harleen *et.al*., ١٩٩٢).

Propionibacterium acnes had lipase activity and this enzyme was very important virulence factor for bacteria associated with acne vulgaris. Gribbon *et.al*., (١٩٩٣) reported that *P. acnes* cell adhesion and adhesion to the internal surfaces of a continuous culture vessel in the presence of the free fatty acid, he also proposed that triglycerides within nascent sebum, which contains no free fatty acid, were partially converted to free fatty acid by *P. acnes* lipase, assisting bacterial adherence and colonization of the sebaceous follicle and induce the development of inflammatory lesions.

P. acnes lipase and the majority of microbial lipase have been reported to be large molecules of aggregates of protein-lipid or protein-protein. A structure of this type explains some of the confecting data on the size and specification of *P. acnes* lipase

as described by various groups who isolated cells from different growth phases (Pablo *et.al*., ١٩٧٤).

This lipase acts as stimulant of the reticuloendothelial system, as an adjuvant to enhance humeral and cell-mediated responses, and as an antitumor agent which modifies the immunological response to tumor (Simth & Woodrull, ١٩٦٨).

Lipase, the extracellular enzyme derived from *P. acnes*, is reported to be responsible for hydrolysis of sebum triglycerides to free fatty acids which have been implicated as both irritants and comedonic agents and which lead to an intensification of the inflammatory process (Freinkel , ١٩٦٩).

Recent reports, however, suggest that the role of this organism may be more complicated (Weeks *et.al*.١٩٧٧). A microphage-specific cytotoxin derived from anaerobic diphtheroids has been reported by Wilkinson, *et.al*.,(١٩٧٣).

The enzyme production can be inhibited by various compounds. Retinol, Tetracycline were effective lipase inhibitors and the degree of inhibition was concentration dependent. In contrast, erythromycin demonstrated no effect upon the lipase similar conclusions were made by Weeks *et.al*.,(١٩٧٧).

Propionibacterium acnes lipase acts directly toward neutrophils in the absence of serum , this lipase is macromolecules and stable at ٥٦^{o}C, indicating that the tertiary structure is probably not involved in its function .

Table (٩) also shows that all isolates had protease production.

Propionibacterium acnes produce follicular proteases, several enzymes that may be important in the inflammatory processes. These protease are produced at optimal pH between ٦-٧ and at optimal temperature between ٣٥-٤٥ C.

The degradation of protein is initiated by proteases secreted by microorganisms. Proteases, are an extra cellular enzymes, of P. acnes aspartic is considered to be a major virulence factor (Beynom & Bond, ١٩٨٩).

Both enzymes had high activities between ٣٥ and ٥٠ ^{o}C and it was seen that these enzymes lost some of their activities when the temperature was higher than ٦٠ ^{o}C

Table (٩)

Detection of enzymes production by *P. acnes* isolates.

Type of enzyme	Isolate No ١	Isolate No ٢	Isolate No ٣
Lipase production	+	+	+
Protease production	+	+	+

٣. ٥ Effect of temperature on *P. acnes* growth

As in table (١٠) the results showed that *P. acnes* grow well at ٢٥,٣٠ and ٣٧ ◦C and that the optimum growth of *P. acne* isolates appeared at temperature ranging from ٣٢-٣٣ ^{o}C under anaerobic

condition and that resembled to the temperature of the skin on body. However, this bacterial growth was inhibited at temperature above ٥٠ ^{o}C.

Temperature can effect on bacterial enzymes through it's influences on the three-dimensional configuration of proteins, thereby affecting the rates of enzymatic activities and as maintained in literature, the optimum temperature for lipase activity is ٣٠-٤٠ ٥C (Ronald, ١٩٩٥).

Acne may increase in cold weather because sebum becomes semiliquid and more collected in hair follicle that will result in increasing *P. acnes* count.

Table (١٠)

Effect of temperature of growth of *Propionibacterium acnes* isolates.

Temperature	Isolate No.١	Isolate No.٢	Isolate No.٣
٢٥٥C	+	+	+
٣٠ ٥C	+	+	+
٣٧ ٥C	+	+	+
٤٠ ٥C	+	+	+
٤٥ ٥C	+	+	+
٥٠ ٥C	-	-	-

٣.٦ Effect of Retinoic acid on *P. acnes* growth

The effect of various Retinoic acid concentration on *P. acnes* growth was studied. It was observed that the addition of Retinoic acid to the growth culture isolates causes decreasing in the growth of these isolates particularly at the concentration above ٢٠ mg/ml, while significant reduction of bacterial growth was markedly observed in the highest concentration spatially at ١٠٠ mg/ml.

Where as the differences between the bacterial growth of the isolates in the same concentration were nearly not detectable.

Retinoids target the microcomedo, which is the precursor of all other acne lesions. Vitamin A has been introduced into the therapy of acne. It decreases markedly the formation of sebum and reduces the population of *Propionibacteria* (Bershad, ٢٠٠١).

Although retinoid does not directly affect *P. acnes* , its inhibitory effect on sebum production leads to an alteration of the follicular microclimate so that an indirect fall in *P. acnes* counts by up to ٣ log cycles can be induced (King *et.al*., ١٩٨٢).

Retinoids down regulate lipid synthesis, decrease late-stage sebocyte differentiation and sebocyte proliferation and reduce sebaceous activity (Guy & Kealey, ١٩٩٧).

Some patients, particularly those with sensitive skin, may experience mild to moderate pruritus, erythema, burning, dryness, or peeling. However, with proper usage and patient education, those local effects can be managed. No longer is topical retinoid irritation seen as a perquisite for topical retinoid efficacy. Topical retinoids offer highly efficacious treatment for both comedonal and inflammatory acne and are the foundation for most successful regimens for the treatment of acne.

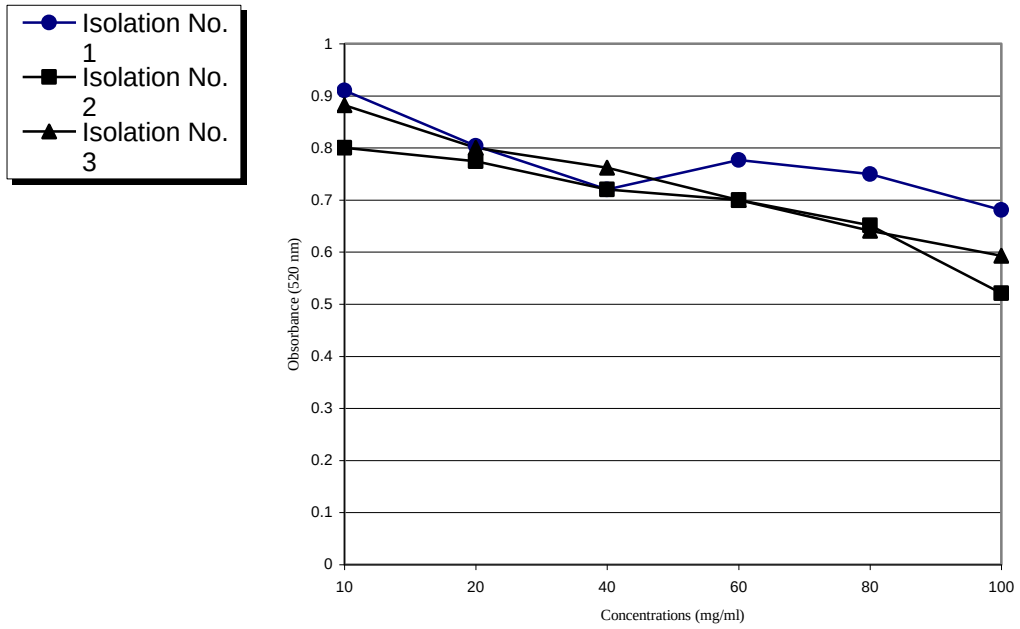
It markedly decrease the formation of sebum and reduces the population of *Propionibacterium.*

Table (١١)

Effect of Retinoic acid on growth of *P. acnes* isolate .

Concentration	Isolate No.١	Isolate No.٢	Isolate No.٣
١٠ mg\ml	٠.٩١٠	٠.٨٠٠	٠.٨٨٢
٢٠ mg\ml	٠.٨٠٤	٠.٧٧٤	٠.٨٠٠
٤٠ mg\ml	٠.٧٢٠	٠.٧٢٠	٠.٧٦٢
٦٠ mg\ml	٠.٧٧٧	٠.٧٠٠	٠.٧٠٠
٨٠ mg\ml	٠.٧٥٠	٠.٦٥١	٠.٦٤١
١٠٠ mg\ml	٠.٦٨١	٠.٥٢١	٠.٥٩٣

Figure (1)
Effect of Retinoic acid on growth of *P. acnes* Isolates



٣.٧ Effect of some antibiotics on *P. acnes* growth

The effect of some antibiotics on *P.acnes* isolates were studed and it was found that the isolates were resistant to erythromycin , fusidic acid , vancomycin , gentamycin whereas the isolates where sensitive to tetracycline ,neomycin, keflexin and chloromphencol.

Antibiogram profiles showed that the isolates gave the same pattern of resistance and sensitivity to antibiotic used in vitro in this study. This may give primary picture on the source of these isolates.

Prior to ١٩٧٦, there were few reports of resistance of *P. acnes* to antibiotics. Since that time , however, *P. acnes* has developed resistance to many of antibiotics used to treat acne .

The prevalence of skin colonization by antibiotic-resistant Propionibacteria in acne patient over a ١٠ years period showed that the preparation of patients carrying strains resistant to one or more commonly used antiacne antibiotics rose steadily in same studies, Ross,(٢٠٠٣) and Coates *et.al*.,(٢٠٠٢) showed that resistance to erythromycin was the most common , and most erythromycin – resistant strains were cross-resistant to clindamysin, and less to tetracycline and deoxycycline .

Tetracycline drugs exert favorable influence on acne, they can inhibit the synthesis of bacterial enzymes, including lipases (Coates *et.al*., ٢٠٠٢).

Other antibiotic agents were also being applied lincomycin, (independence on the limits for tetracycline) indicated and according to the verified therapy used by various centers (Leyden, ٢٠٠١) .

Table (١٢)

Sensitivity of P. acnes isolate to deferent antibiotics

Antibiotics	Isolate No.١	Isolate No.٢	Isolate No.٣
Erythromycin	+	+	+
Tetracycline	-	-	-
Fusidic acid	+	+	+
Vencomycin	+	+	+
Neomycin	–	–	–
Chloromphenecol	–	–	–
Gentamycin	+	+	+
Lincomycin	+	+	+
Kephlexin	–	–	–
Deoxycyclin	–	–	–

(+) resistant (-) sensitive

In ١٩٧٦ researchers found no *P. acnes* resistant to antibiotics . In ١٩٩١, surveys found ٣٨٪ of acne patients carried bacterial strains resistant to one or more antibiotics, ٢٦.٥٪ carried erythromycin-

resistant strain , and ١٣٪ carried tetracycline-resistant, resistant to minocycline was less than ١٪ .

Tetracycline interfere with protein synthesis by combining with bacterial ribosome. It is bacteriostatic that the first line in acne treatment specially with sever cases that have direct effecting in reducing free fatty acid and inhibiting its lypolytic activity. Tetracyclin resistance remains a clinically significant determinant to the utility of tetracycline and deoxycyclin and other commercially available tetracycline. There are two measurement mechanisms of tetracycline resistance : efflux and ribosome production. Both mechanisms have been described in gram positive and gram negative bacteria either separately or together, with the ribosome production generally more common in gram positive and efflux in gram negative (Robberts, ١٩٩٦).

Tetracycline also inhibits lipase activity in *S. aureus* that leads to decrease of all bacteria types in acnes that led to reduce inflammatory disease (Champion, *et.al*., ١٩٩٨)

Tetracycline is the most common drug that is as germicidal agent and decrease free fatty acids in skin .

Also erythromycin and clindamycin used in decreasing *P. acnes were* counted while chloromphenicol was used as anti-inflammatory in addition to antibacterial (Cunliffe, ١٩٨٢) .

Doxycycline is a form of tetracyclines group and has the same effectes with acne in addition to that it is lipophilic which means it may collect in follicles (Laurnce *et.al*., ١٩٩٧)

Clindamycin is the second choice in acne treatment especially with moderate and sever acne when the bacteria is resistant to other antibiotics.

Also, it has bacterostatics and lipase inhibition effect, clindamycin has anti comedonal effect. The side effect of clindamycin use is diarrhea and pseudomembranous colitis (komagato *et.al*., ١٩٩٨)

Moreover , erythromycine may also be used in treatment when other antibiotics fail in treatment of acne especially with pregnant women and breast feeding women, the batter treatment with this drug when it combines with topical benzol peroxide (Laurance *et.al*., ١٩٩٧) .

Farthermore, neomycin , fusidic acid and gentamycin can be used in treatment of acne vulgaris. Fusidic acid can also be used in treatment of some skin infection other than acne vulgaris (Greenwood, ١٩٩٦).

٣.٨ Immune response in acne

The results of differential WBC's count are shown in table (١٣) , and it was found that all the cells count were in normal values except Monocytes which had increased at high levels

where ١٣ patients were seen to have high levels (> ٧) of Monocytes counts . This result was observed by Burkhart , *et.al.,* (١٩٩٩) who reported that the inflammation in acne vulgaris might be mainly induced by an immunological reaction to *P.acnes* . Chemotactive substances released by bacteria attract cells of the immune system such as Monocytes and Lymphocytes (Roitt, *et.al.,* ٢٠٠١).

Propionibacterium acnes produce soluble factors that are able to activate immune cells with consecutive secretion of variouse proinflammatory cytokines (IL-٨ Tumer necrosis factor , IL-١) (Dasgupta, ١٩٩٢) (Chen *et.al.,* ٢٠٠٢).

Kim *et.al.,*(٢٠٠٠) showed that Lymphocyte stimulation by *P. acnes* significantly increases in patients with nodulocystic acne.

Parslow *et.al.,* (٢٠٠١) reported that phagocyte activity for professional phagocytes such as Monocytes in peripheral blood was usually affected in chronic bacterial infections which usually appeared as significant difference in phagocytes present in blood films.

Ashbee *et.al.,* (١٩٩٧) reported that patients with severs acne had significantly higher levels of specific immunoglobulin class especially IgG and IgM in peripheral blood and complement fixing antibodies to *P. acnes* .

This correlates with the results of this study that there is significant value in IgG concentration in patient's peripheral

blood also IgM concentrations but in lesser value compared with normal persons.

Antibodies to *P. acnes* may be involved in the pathogensis of acne vulgaris but there is evidence to suggest a role for antibodies to *P. acnes* exocellar enzymes in the initiation of inflammatory response in acne (Ingham et.al., ١٩٨٧).

So *P. acnes* influence both cellular and humeral components of the immune system (Kurokawa *et.al.*, ١٩٩٩). They are able to persist in macrophages and increase chemotaxis of poly-morphnuclear leukocytes. They are also cytotoxically active are able to activate the complement in an alternative way and bring about the hypersensitivity of early or late type (Auffret. ٢٠٠٠).

Table (١٣)
Diffrential WBCs count in Normals

No.	Sex	Age	Neutrophiles	Lymphocytes	Monocytes	Esionophyles
١	**M**	١٧	٧٣	٢٥	١	١
٢	**M**	١٩	٦٨	٢٩	٢	١
٣	**M**	٢٠	٦٥	٣١	٢	٢
٤	**M**	٢١	٦٢	٣٠	٦	٢
٥	**M**	٢١	٧٤	٢٣	١	٢
٦	**M**	٢١	٦٦	٣٢	١	١
٧	**M**	٢١	٦٠	٣٠	٨	٢
٨	**M**	٢٢	٦٢	٢٩	٨	١
٩	**M**	٢٢	٧٠	٢٣	٥	٢
١٠	**F**	١٨	٦٨	٢٨	٢	٢
١١	**F**	١٨	٦٩	٢٧	٢	٢
١٢	**F**	١٨	٦٥	٣٢	٢	١
١٣	**F**	١٩	٦٩	٢٨	٢	١
١٤	**F**	٢٠	٧٢	٢٥	١	٢
١٥	**F**	٢٠	٧٠	٢٩	١	٠
١٦	**F**	٢٠	٧٠	٢٨	١	١
١٧	**F**	٢٠	٦٧	٢٨	٥	٠
١٨	**F**	٢١	٦٨	٣٠	١	١
١٩	**F**	٢١	٦٨	٢٨	٣	١
٢٠	**F**	٢٣	٦٩	٢٨	١	٢

Table (١٤)
Diffrential WBCs count in Patients

No.	Sex	Age	Neutrophiles	Lymphocytes	Monocytes	Esionophyles
١	M	١٧	٥٢	٢٦	٢٠	٢
٢	M	١٧	٤٤	٣٨	١٧	١
٣	M	١٨	٤٢	٤١	١٦	١
٤	M	١٩	٤٥	٣٨	١٦	١
٥	M	٢٠	٥٧	٣٥	٦	٢
٦	M	٢٠	٦٣	٢٩	٧	١
٧	M	٢٠	٤٦	٣٢	٨	٤
٨	F	١٥	٥٢	٢٨	١٨	٢
٩	F	١٨	٤٤	٤٠	١٥	١
١٠	F	١٩	٥٣	٣٩	٨	٠
١١	F	١٩	٤٢	٤١	١٥	٢
١٢	F	١٩	٦٤	٣٢	٣	١
١٣	F	٢٠	٢٥	٣٠	٩	٢
١٤	F	٢٠	٦٢	٢٧	٩	٢
١٥	F	٢٠	٦٠	٣٠	٨	٢
١٦	F	٢٠	٤٥	٢٩	٢٤	٢
١٧	F	٢٠	٥٤	٣٠	١٥	١
١٨	F	٢١	٤٩	٣٧	١٣	١
١٩	F	٢١	٥٢	٣٤	١٣	١
٢٠	F	٢٤	٧٢	٢٤	٣	١

Figure (3)
Mean of Differential WBCs count of Normal and Acne patients

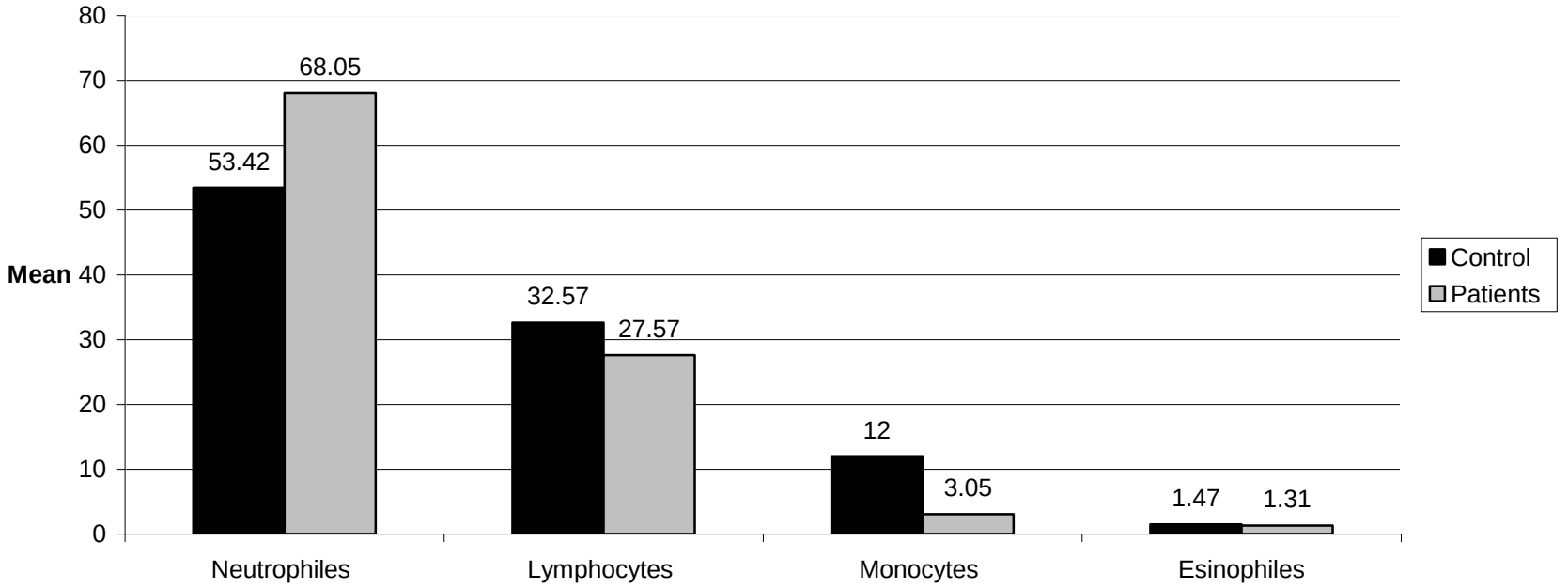


Table (١٥)
concentrations of IgG, IgM (mg/dl) in normal and acne in Male patients

No.	Normal				Patients			
	Sex	Age	IgG (mg/dl)	IgM (mg/dl)	Sex	Age	IgG (mg/dl)	IgM (mg/dl)
١	M	١٧	١٠٣١.٢	٧٧.٣	M	١٧	١١٣٣.٧	١٤٧.٤
٢	M	١٩	٧٩٠.١	١١٦.٤	M	١٧	١١٦٨.٢	١٠٨.٩
٣	M	٢٠	٩٣٢.٢	١٥٣.٨	M	١٨	١٦٩٧	٢٠٩.٥
٤	M	٢١	٩٨١.٨	٢٠٩.٥	M	١٩	٢١٤٢.١	١٨٠.٩
٥	M	٢١	٦١٢	١٤٠.٩	M	٢٠	٢١٤٢.١	١٦٧.١
٦	M	٢١	٦٥٥.٧	٦٧.١	M	٢٠	١٥١٩	١٧٨.٩
٧	M	٢١	٩٣٢.٢	٩٣.٤	M	٢٠	٩٨١.٣	١٣٤.٦
٨	M	٢٢	٥٧٠.٤	١٧٤				
٩	M	٢٢	٦١٢.٦	١٤٠.٩				

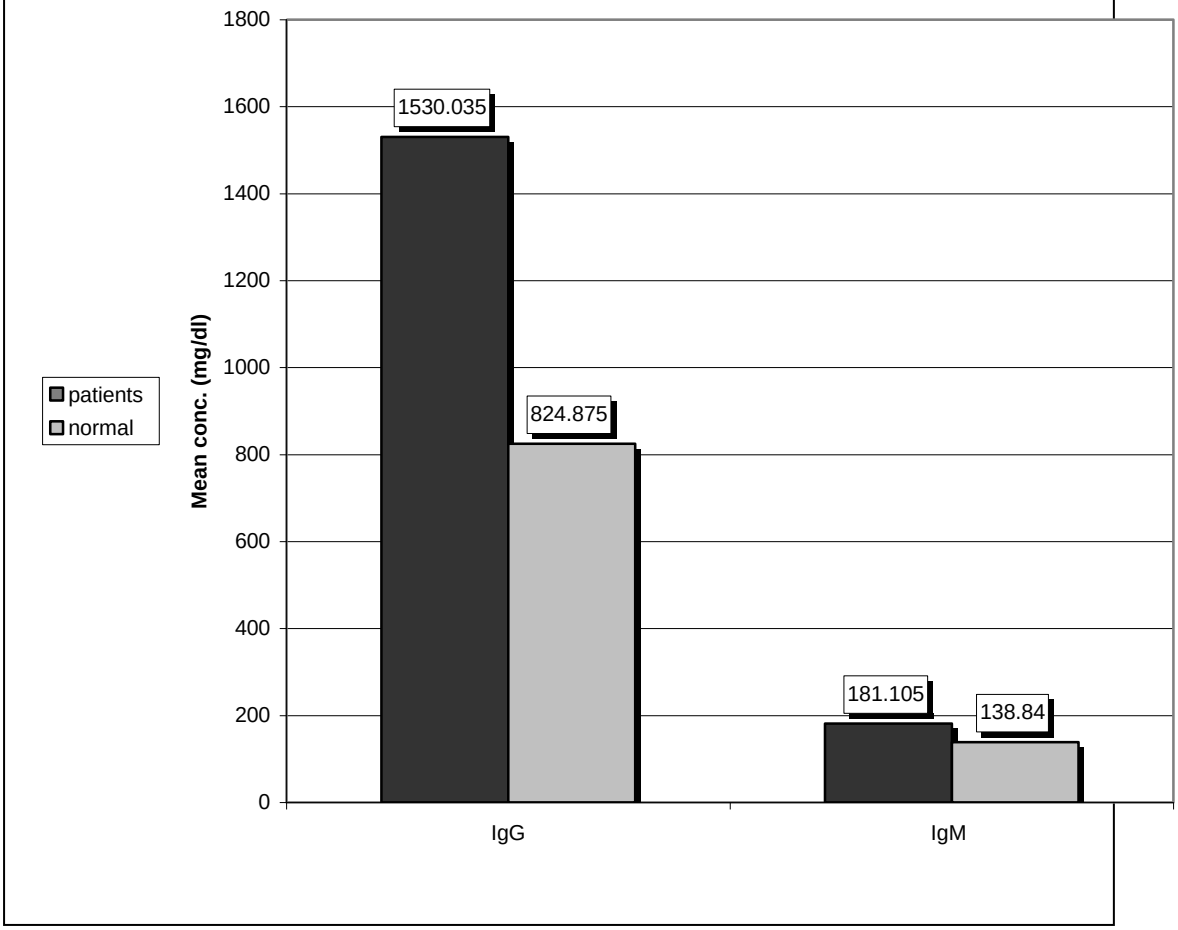
The total number of male patient was ٧, while normal male was ٩

Table (١٦)
concentrations of IgG, IgM (mg/dl) in normal and acne in Female patients

No.	Normal				Patients			
	Sex	Age	IgG (mg/dl)	IgM (mg/dl)	Sex	Age	IgG (mg/dl)	IgM (mg/dl)
١	F	١٨	٨٨٤	١٤٧.٣	F	١٥	١٩٤٦.٢	٢٣٩.٨
٢	F	١٨	٩٣٢.٢	٩٣.٤	F	١٨	٢٢٠٩.١	٢٨٠
٣	F	١٨	٨٣٦.٧	١١٦.٤	F	١٩	١٢٩٣.٧	١٣٤.٦
٤	F	١٩	٩٨١.٣	١٦٧.١	F	١٩	١٩٤٦.٢	٢١٧
٥	F	٢٠	٩٨١.٣	١٨٠.٩	F	١٩	١٦٣٦.٨	١٨٧.٩
٦	F	٢٠	١٠٨٢	٩٣.٤	F	٢٠	١٢٣٩.٥	١٥٣.٨
٧	F	٢٠	٧٤٤.٥	٩٩	F	٢٠	١١٣٣.٧	١٨٧.٩
٨	F	٢٠	٧٤٤.٥	١٥٣.٨	F	٢٠	١٣٤٨.٧	١٤٧.٣
٩	F	٢١	٧٩٠.١	١٨٧.٩	F	٢٠	١١٨٦.٢	٢٠٢.٢
١٠	F	٢١	٦١٢.٦	٢١٧	F	٢٠	١٧٥٨	٢٧١.٨
١١	F	٢٣	٧٩٠.١	١٤٧.٣	F	٢١	١١٣٣.٧	٦٧.١
١٢					F	٢١	١٣٤٨.٧	٢٢٤.٥
١٣					F	٢٤	١٦٣٦.٨	١٨٠.٩

The total number of female patient was ١٣, while normal female was ١١

Figure (4)
Mean concentrations of IgG, IgM (mg/dl) in normal and acne patients



٤.١ Conclusions:

١- Acne vulgaris infect female more than male especially in adolescent age.

٢- *Propionibactrium acnes* have many virulence factors such as capsule, CFA/III, lipase and protease.

٣- Acne treated with either systemic or optical treatment.

٤- Retinoic acid effect on bacterial growth especially in high concentration.

٥- Presence of *Propionibactrium acnes* stimulates both cellular and humeral immune response in acne patients.

٤.٢ Recommendation

According to the results obtained in the present study, we can recommened the follwing :

١- Study of *Propionibacterium acnes* in disease other than acne vulgaris.

٢- Sudy of molecular basis of *Propionibacterium acnes* isolated from acne patients.

٣- Evade of using both systemic and tropical antibiotics which can effect on skin normal flora and then colonization of bacteria.

٤- Investigation of new generations of antibiotics for treatment of *Prropionibacterium acnes* infections.

Table ( )
concentrations of IgG, IgM (mg/dl) in normal and acne in Male patients

No.	Normal				Patients			
	Sex	Age	IgG (mg/dl)	IgM (mg/dl)	Sex	Age	IgG (mg/dl)	IgM (mg/dl)
١	M	١٧	١٠٣١.٢	٧٧.٣	M	١٧	١١٣٣.٧	١٤٧.٤
٢	M	١٩	٧٩٠.١	١١٦.٤	M	١٧	١١٦٨.٢	١٠٨.٩
٣	M	٢٠	٩٣٢.٢	١٥٣.٨	M	١٨	١٦٩٧	٢٠٩.٥
٤	M	٢١	٩٨١.٨	٢٠٩.٥	M	١٩	٢١٤٢.١	١٨٠.٩
٥	M	٢١	٦١٢	١٤٠.٩	M	٢٠	٢١٤٢.١	١٦٧.١
٦	M	٢١	٦٥٥.٧	٦٧.١	M	٢٠	١٥١٩	١٧٨.٩
٧	M	٢١	٩٣٢.٢	٩٣.٤	M	٢٠	٩٨١.٣	١٣٤.٦
٨	M	٢٢	٥٧٠.٤	١٧٤				
٩	M	٢٢	٦١٢.٦	١٤٠.٩				

The total number of male patient was ٧, while normal male was ٩

Table ( )
concentrations of IgG, IgM (mg/dl) in normal and acne in Female patients

No.	Normal				Patients			
	Sex	Age	IgG (mg/dl)	IgM (mg/dl)	Sex	Age	IgG (mg/dl)	IgM (mg/dl)
١	F	١٨	٨٨٤	١٤٧.٣	F	١٥	١٩٤٦.٢	٢٣٩.٨
٢	F	١٨	٩٣٢.٢	٩٣.٤	F	١٨	٢٢٠٩.١	٢٨٠
٣	F	١٨	٨٣٦.٧	١١٦.٤	F	١٩	١٢٩٣.٧	١٣٤.٦
٤	F	١٩	٩٨١.٣	١٦٧.١	F	١٩	١٩٤٦.٢	٢١٧
٥	F	٢٠	٩٨١.٣	١٨٠.٩	F	١٩	١٦٣٦.٨	١٨٧.٩
٦	F	٢٠	١٠٨٢	٩٣.٤	F	٢٠	١٢٣٩.٥	١٥٣.٨
٧	F	٢٠	٧٤٤.٥	٩٩	F	٢٠	١١٣٣.٧	١٨٧.٩
٨	F	٢٠	٧٤٤.٥	١٥٣.٨	F	٢٠	١٣٤٨.٧	١٤٧.٣
٩	F	٢١	٧٩٠.١	١٨٧.٩	F	٢٠	١١٨٦.٢	٢٠٢.٢
١٠	F	٢١	٦١٢.٦	٢١٧	F	٢٠	١٧٥٨	٢٧١.٨
١١	F	٢٣	٧٩٠.١	١٤٧.٣	F	٢١	١١٣٣.٧	٦٧.١
١٢					F	٢١	١٣٤٨.٧	٢٢٤.٥
١٣					F	٢٤	١٦٣٦.٨	١٨٠.٩

The total number of female patient was ١٣, while normal female was ١١

Table ()
Diffrential WBCs count in Normals

No.	Sex	Age	Neutrophiles	Lymphocytes	Monocytes	Esionophyles
١	M	١٧	٧٣	٢٥	١	١
٢	M	١٩	٦٨	٢٩	٢	١
٣	M	٢٠	٦٥	٣١	٢	٢
٤	M	٢١	٦٢	٣٠	٦	٢
٥	M	٢١	٧٤	٢٣	١	٢
٦	M	٢١	٦٦	٣٢	١	١
٧	M	٢١	٦٠	٢٩	٩	٢
٨	M	٢٢	٦٢	٢٩	٨	١
٩	M	٢٢	٧٠	٢٣	٥	٢
١٠	F	١٨	٦٨	٢٨	٢	٢
١١	F	١٨	٦٩	٢٧	٢	٢
١٢	F	١٨	٦٥	٣٢	٢	١
١٣	F	١٩	٦٩	٢٨	٢	١
١٤	F	٢٠	٧٢	٢٥	١	٢
١٥	F	٢٠	٧٠	٢٩	١	٠
١٦	F	٢٠	٧٠	٢٨	١	١
١٧	F	٢٠	٦٧	٢٠	١٣	٠
١٨	F	٢١	٦٨	٣٠	١	١
١٩	F	٢١	٦٨	٢٨	٣	١
٢٠	F	٢٣	٦٩	٢٨	١	٢

Table ()
Diffrential WBCs count in Patients

No.	Sex	Age	Neutrophiles	Lymphocytes	Monocytes	Esionophyles
١	M	١٧	٥٢	٢٦	٢٠	٢
٢	M	١٧	٤٤	٣٨	١٧	١
٣	M	١٨	٤٢	٤١	١٦	١
٤	M	١٩	٤٥	٣٨	١٦	١
٥	M	٢٠	٥٧	٣٥	٦	٢
٦	M	٢٠	٦٣	٢٩	٧	١
٧	M	٢٠	٤٦	٣٢	٨	٤
٨	F	١٥	٥٢	٢٨	١٨	٢
٩	F	١٨	٤٤	٤٠	١٥	١
١٠	F	١٩	٥٣	٣٩	٨	٠
١١	F	١٩	٤٢	٤١	١٥	٢
١٢	F	١٩	٦٤	٣٢	٣	١
١٣	F	٢٠	٢٥	٣٠	٩	٢
١٤	F	٢٠	٦٢	٢٧	٩	٢
١٥	F	٢٠	٦٠	٣٠	٨	٢
١٦	F	٢٠	٤٥	٢٩	٢٤	٢
١٧	F	٢٠	٥٤	٣٠	١٥	١
١٨	F	٢١	٤٩	٣٧	١٣	١
١٩	F	٢١	٥٢	٣٤	١٣	١
٢٠	F	٢٤	٧٢	٢٤	٣	١

Immune response in acne

The cellular infiltrate around inflamed acne lesions is characteristic of a delayed type hyperseitivity response to one or more antigens, not necessarily propionibacterial. Damage to the follicle wall will allow non bacterial antigens to escape into the dermis. The immune response in acne, particularly severe acne is similar to that seen in tuberculosis. Like mycobacteria P. acnes exhibit potent adjuvant activity and non specifically up regulate macrophage functions (Eady & Richard, ٢٠٠١).

Table ( ) presents twenty acne patients during adolescence in both sex and there differential WBC's count in compared with twenty control subjects also in adolescence age in compared with twenty control subjects also in adolescence age in table ( ) . The results showed highly significant reading in both lymphocytes and Monocytes in acne patients blood in compared with control , also figure ( ) in sure this results by increase the mean in both Lymphocytes (٣٣) and Monocytes (١٢.١٥) in acne patients serum in compared with (٢٧.٧)and (٣.٢٠) respectively in control serum.

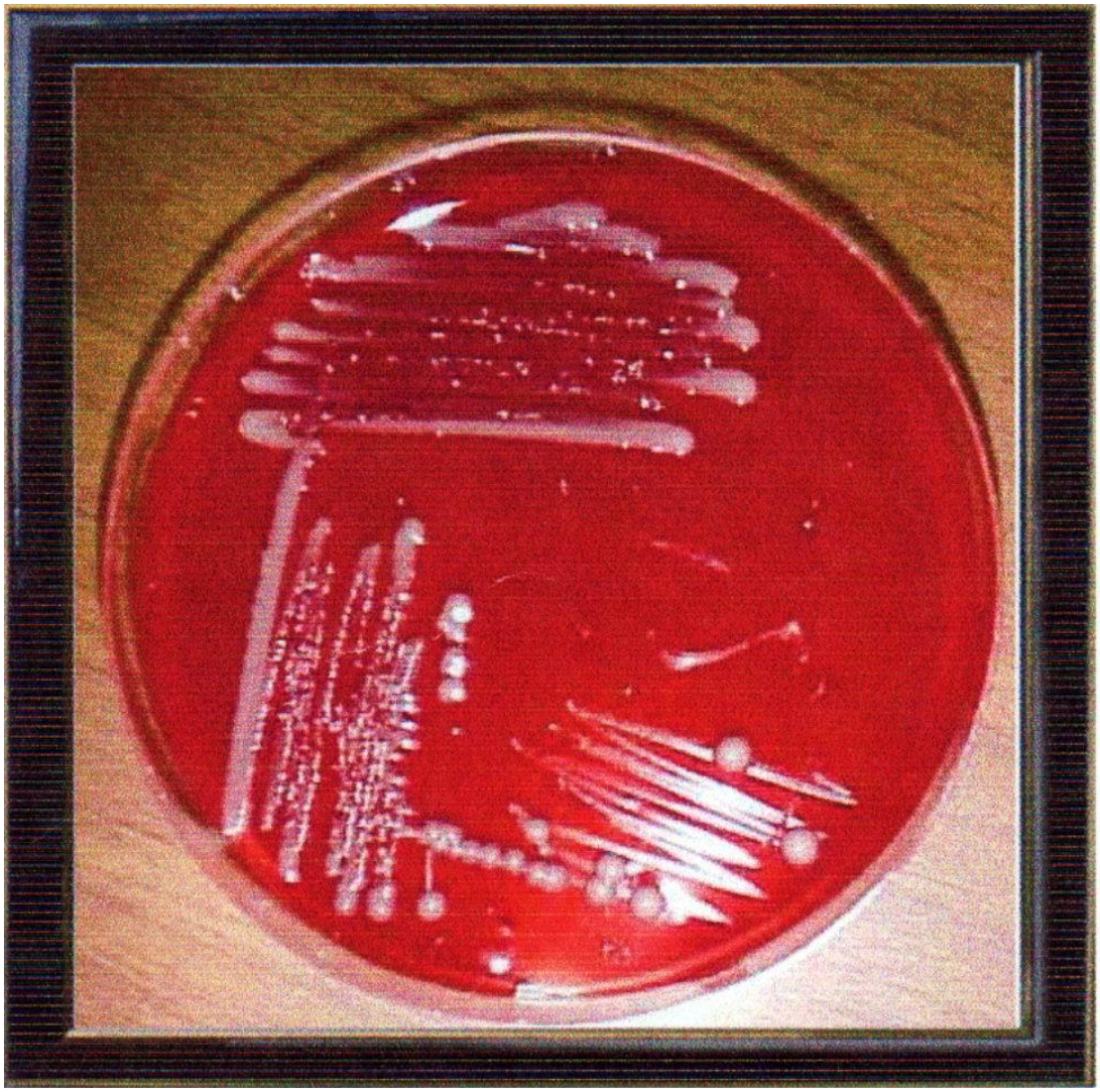
This may be explained by fact inflammation in acne vulgaris were due to immunological reaction of P. acnes. Chemotactive substances released by bacteria attack against cells of the immune system such as neutrophils, monocytes and lymphocytes (Burkhart *et.al.*, ١٩٩٩).

Parslow et.al. (٢٠٠١) reported that phagocyte activity for professional phagocytes such as monocytes in peripheral blood is usually affected in chronic bacterial infections which usually appear as significant difference in phagocytes present in blood films.

The ability of *P. acnes* to induce specific immunoglobulin class specially IgG and IgM in peripheral blood also obtained from acne patients and healthy subjects , were also analyzed. The concentration of

IgG were significantly higher in sever chronic acne compared with control (١٥٣٠ .٩٣ mg/dl) and (١١٠٨.٧١ mg/dl) respectively. Also concentration of IgM (١٨١.٥٥ mg/dl) in acne patients compared with healthy person (١٣٨.٨٤).

Antibodies to *P. acnes* may be involved in the pathogenesis of acne vulgaris (Ashbee *et.al.*,١٩٩٧) but there is evidence to suggest a role for antibodies to P. acnes exocellular enzymes in the initiation of inflammatory response in acne (Ingham et.al., ١٩٨٧).



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