ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at: www.mjotu.com

العراقية
المجلات الاكاديمية العلمية
IRAQI
Academic Scientific Journals

The Efficacy of Oral Isotretinion in Reduction of Lipase Enzyme Activity of Cutibacterium acnes in Acne Vulgaris Iraqian Patients

Wakas S. Mahmood *(1)

Nameer K. Qasim (2)

Nahla G. Abdulmajeed (3)

(1) Department of Biology
College of Science
Tikrit University
Salahaldeen
Iraq

(2) College of Medicine
Ibn-Sina university of medical & pharmaceutical sciences
Baghdad
Iraq

(3) Immunological Laboratory Center
Medical City
Baghdad
Iraq

Keywords:

Cutibacterium acnes,
Acne vulgaris,
Isotretinion,
Lipase enzyme,
Oral Isotretinion.

ARTICLE INFO

Article history:

Received	01 Oct 2018
Accepted	01 Dec 2018
Available online	01 June 2019

ABSTRACT:

Background Acne vulgaris is one of the top three most common encountered dermatological problems worldwide in both primary and secondary care. A relation between acne vulgaris and Cutibacterium acnes(Propinoibacterium acnes) has long been suggested. A lipase enzyme secreted by this microorganism to metabolize sebum and the resulting metabolites evoke inflammation in human skin. The current study is conducted to assess the oral isotretinion treatment for acne vulgaris patients and understanding of C. acnes' pathogenicity; via the lipase enzyme.

Patients and Methods:50 patients with acne vulgaris seen in outpatient clinic of dermatology center in Baghdad during the period 1/9/2017 to 1/8/2018. The mean ages was 22±16-36 years. From the total of 50 patients,36(72%) of patients were females and 14(28%) were males. Furthermore 43(86%) of cases have previous family history of acne. Fifty samples from acne lesions were collected before oral isotretinion therapy, only forty-five samples were collected after oral isotretinion therapy. The diagnosis of patients were done by the specialist dermatologist, swabs and needle aspiration were collected from papules, pustules and nodulocystic lesions by the physician. The specimens were transported to the laboratory by using thioglycollate broth. The samples were cultured on Brucella blood agar with furazolidone in an anaerobic conditions, only C. acnes' were grown. C. acnes isolates were cultured on spirit blue agar at 37 °C for 7days under anaerobic condition, to identify the lipase activity.

Results: All isolates of C. acnes were lipase positive before oral isotretinion therapy. The degree of lipase enzyme significantly decreased after treatment with oral isotretinion in 44(98%) of isolates but 1(2%) of the cases was not. These results indicates that lipase was the common virulent enzyme which produced by C. acnes isolated that isolated from acne lesions. All of the clinical isolates were demonstrating strong lipase production before treatment .

Conclusion: Great emphasis should be done on choosing the drug that is used to inhibit or decreases the sebum production and hyperkeratinization of pilosebaceous follicle unit activity rather than hypercolonization of C. acnes. So, using oral isotretionion will prevent the optimal environment conditions for the production of lipase by this bacterium, and subsequently oral isotretinoin therapy gives good improvement response.

DOI: http://dx.doi.org/10.25130/mjotu.25.01.09

*Corresponding author E mail : wakas_saadi81@yahoo.com

Introduction

Acne vulgaris affecting more than 85% of adolescents,and can also persist into adulthood (1). Acne vulgaris is the most common disease of pilosebaceous unit characterized by non-inflammatory lesions (open and closed comedons), and inflammatory lesions (papules, pustules and nodules) (2). The pathogenesis of acne is multifactorial, including sebum production, hyper-keratinization of infundibulium due to release of pilosebaceous content to surrounding dermis, hypercolonization of *Cutibacterium acnes*, and inflammation (3).

The Gram-positive anaerobic *C. acnes* is responsible for the development of acne vulgaris when overgrowth in pilosebaceous follicle, although this bacterium is regarded a flora inhabiting the sebaceous glands. One major virulence factor of the *C. acnes* is a lipase enzyme that split triglycerides in sebum to release free fatty acids (4). *C. acnes* use the lipids in sebum for growth and when the sebum production increases, more nutrients are available and the density of *C. acnes* bacteria can increase (5).

The sebaceous gland cell in pilosebaceous unit is a key playerin the pathogenesis of acne (6). The change in environment-provided carbon sources, and low oxygen tension is reflected in the activation of lipases genes. The two lipases acquired by *C. acnes* present in human skin, triacylglycerol lipase and lysophospholipase, that facilitate *C. acnes* survival in the harsh environment of sebaceous follicles (7). Therefore, the oxygen tension in follicles may well affect the interaction of the Cutibacteria with their environment (8).

Cutibacterium acnes colonization in pilosebaceous sites is explained by the multitude of lipases found in its genome, which it uses to acquire nutrients from lipid-enriched sebum (9).The anaerobic and lipid-enriched conditions within the pilosebaceous unit give an optimal microenvironment for *C. acnes* growth (10). Most importantly, lipase enzyme derived from the *C. acnes* is able to convert sebum into free fatty acids which may be lead to the skin irritation (11). Colonization of the pilosebaceous follicle unit by *C. acnes* is a main factor for the inflammatory response in acne vulgaris; so ,*C. acnes* has been a major target of treatment in acne vulgaris (12).

There is no ideal treatment for acne, although a suitable regimen for reducing lesions can be found for most patients. Oral isotretinoin is the most effective therapy in acne vulgaris. The aim of treatment of acne vulgaris is to prevent long –term

complications. Improved understanding about the role of *C. acnes* in acne should help scientists in designing efficacious treatment strategies (13).

The aim of this study is to assess the effect of oral isotretinion as a major treatment of acne vulgaris, by inhibiting *C. acnes* lipase enzyme as a major virulence factor of this bacterium. And give new concept that focus on the relationship between acne condition (i.e. microenviroment) and *C. acnes* hypercolonioization, by directly or indirectly effect of oral isotretinoin.

Materials and Method:

The work was done in the laboratories of department of microbiology / College of Dentistry, University of Ibn-Sina, Baghdad city, where these devices, tools and materials are available, whether glasses or chemicals in their laboratories.

This study is a prospective study one and conducted during the period September 2017 to August 2018. Fifty patients with acne were recruited of dermatology clinics (Al-Baidhaa out clinic center/Baghdad city). The diagnosis of acne was made by specialist dermatologists. All patients have received 0.5mg-1mg/kg/day of oral isotretinoin (14). All patients have been sampled before treatment, and 45(90%) of patients were sampled after 8 weeks of therapy whereas other cases was default. Samples were collected aseptically from the most representative site of three kinds of acne lesions (papule, pustule, and nodulocyst) from each patient. For acne lesion sampling, the surface was wiped with alcohol and a small hole was made aseptically in the centre of the lesion with a needle. Then, the site was gently expressed by an extractor, and the contents were collected on a sterile swab stick. The samples were placed in thioglycollate broth medium and subsequently streaked on brucella blood plates. The antibiotic furazolidone was added to the base modified medium in order to eliminate staphylococci bacteria and thus render the medium selective to *C. acnes*, then incubated in anaerobic jars (15).

A challenge in the culture-based diagnosis of *C. acnes* is that it grows slowly and favored anaerobic conditions (16). After incubation for seven days anaerobically, on brucella blood agar plates are examined (17). Cutibacteria were identified to species level by performing a Gram stain, colony morphology, indole test, nitrate test and catalase test (18).

Virulence factor, namely lipase was detected as reported by Grech, and McDowell, *et al,* (18, 19). This

test was used to determine the ability of bacterial isolates to excrete extracellular enzyme lipase that capable of degrading the lipid. Briefly, *C. acnes* isolates were cultured on supplemented brain heart infusion agar at 37 °C for 7 days under anaerobic condition. After incubation, a single colony of the bacteria was streaked over the Spirit Blue agar media surface to screen for lipase activity. The plates were than incubated for 7 days at 37 °C under anaerobic condition. The isolates producing clear zone or the opalescent zone around the colonies were identified as lipase positive (11).

Results and Discussion:

Many patients admitted into the outpatient clinic of dermatological care center in Baghdad city with acne vulgaris. Only 50 patient were recruited in this study depending on the clinical diagnosis and investigations. After sample collection, all patients will received oral isotretinoin for 8 weeks. After this period only 45 patients returns into the clinic centre and samples have been collected again. From 50 cases of acne vulgaris, 36 (72%) of patients were females, while 14(28%) were males with female to male ratio of about (4:1). Also, The mean ages was 22±16-36 years. The number of teens was 34(68%), whereas, young adults were 11(22%), and the adults were 5(10%) patients only, and 43(86%) of cases have heredity history.

After identification, all isolates gives positive results for *C. acnes* whether before or after oral isotretinoin treatments, see figures(1, 2, and 3).

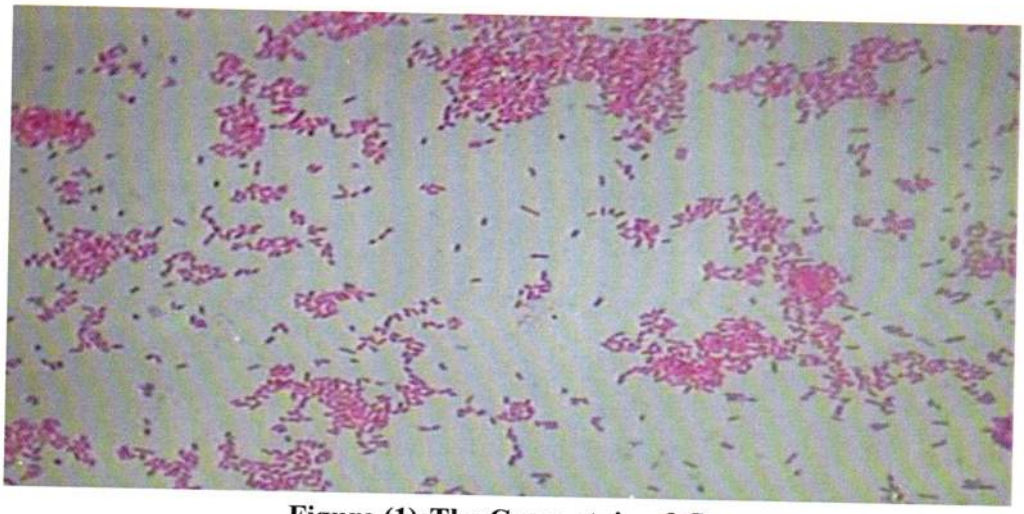

Figure (1):The Gram stain of *C.acnes*

Figure (2):The growth of *C. acnes* on thioglycollate broth

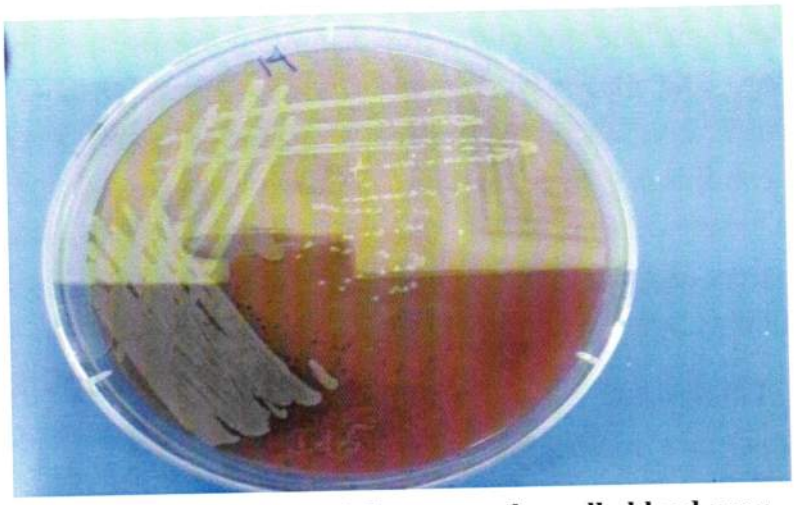

Figure (3):The growth of *C. acnes* on brucella blood agar .

Screening of enzyme production showed that all of the isolates possessed lipase activity before treatment, while, 44(98%) isolates were negative for lipase activity and only, 1(2%) of isolates was exhibiting lipase activity, after treatment, see (figure 4), also see figures(5 and 6).

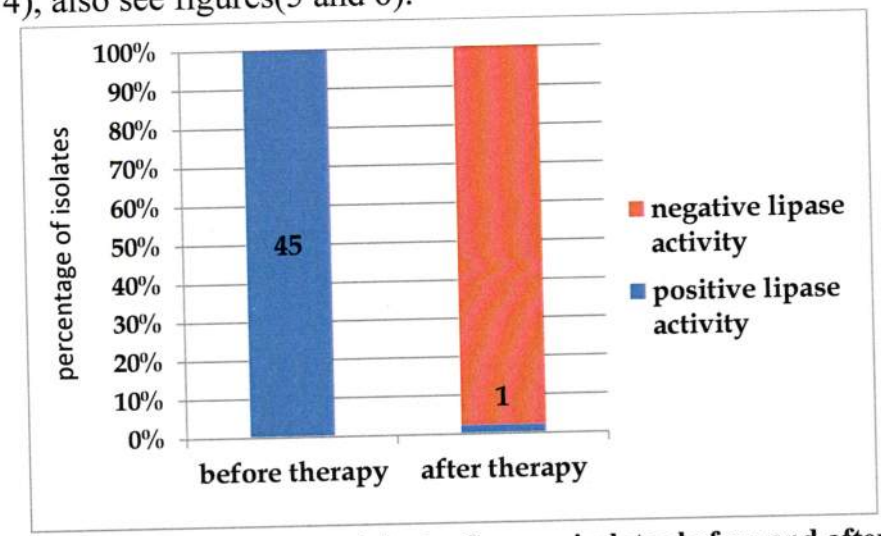

Figure (4): frequency of lipase activity in *C. acnes* isolates before and after therapy

These results indicated that lipase was the common virulence factor which is produced by C. acnes isolated from acne lesions. All of the clinical isolates of C. acnes which demonstrated strong lipase production. Whereas after the treatment with oral isotretinoin, most isolates loses the ability to produce lipase enzyme. In the present study, we found, the important role of isotretinoin as agent inhibits the lipase activity of C. acnes, that related with the strong activity of oral isotretinoin as a good treatment which inhibit the most important virulence factor of C. acnes (11, 4). The production of lipases and proteases by C. acnes grown on artificial sebum (7-day old cultures in order to have a sufficient cell count) was assessed (20). Additionally, oxidized lipids in sebum cell can stimulate production of inflammatory mediators, which further drives the inflammatory process (21.(

The pathogenicity of Cutibacteria is thought to be due to, first, the production of exocellular enzymes and exocellular products, which could act as virulence determinants, and, second, on the bacterium interaction with the immune system (22). Extracellular lipase work as virulence factor in the pathogenesis of C. acnes. Therefore, lipase inhibition affected on the free fatty acid production that could leads to reduction in the growth of C. acnes. Therefore, concerning the role of lipase enzyme on pathogenicity of C. acnes, the effects of isotertinoin on lipase production is clear (11).

Higaki, et al, showed a correlation between the severity degree of acne and lipase activity. C. acnes secrets lipase which interact with molecular oxygen, that produced reduced oxygen species and free radicals causing keratinocyte damage and inflammation (23).

The lipase secreted by C. acnes metabolize sebum and the resulting metabolites evoke inflammation in human skin (4).Studies were suggested that drugs were used either to increase or to decrease sebum production, resulting in a concomitant increase or decrease in the numbers of Cutibacteria (24), which helped to

strength our results and also strength our concept further .

Retinoids have a unique action reducing formation of acne precursor lesions and limiting development of new lesions (25). The feature of oral isotretinoin that favor by the clinician's on the other drugs is the targets of oral isotretinoin. Oral isotretinoin is the most effective agent available for the treatment of acne, as the drug targets all four pathogenic mechanisms of acne. Oral isotretinoin is only the drug that effect on sebum production, altered keratinization, C. acnes proliferation and inflammation (26). Several studies have shown that isotretinoin effectively decreases sebum production, the number of acne lesions, and acne scarring (27). Isotretinoin is used to treat not only severe, cystic and scarring acne vulgaris but also refractory disease. It reduces sebaceous gland size and secretory activity, comedone formation and follicular colonization of C. acnes (28).

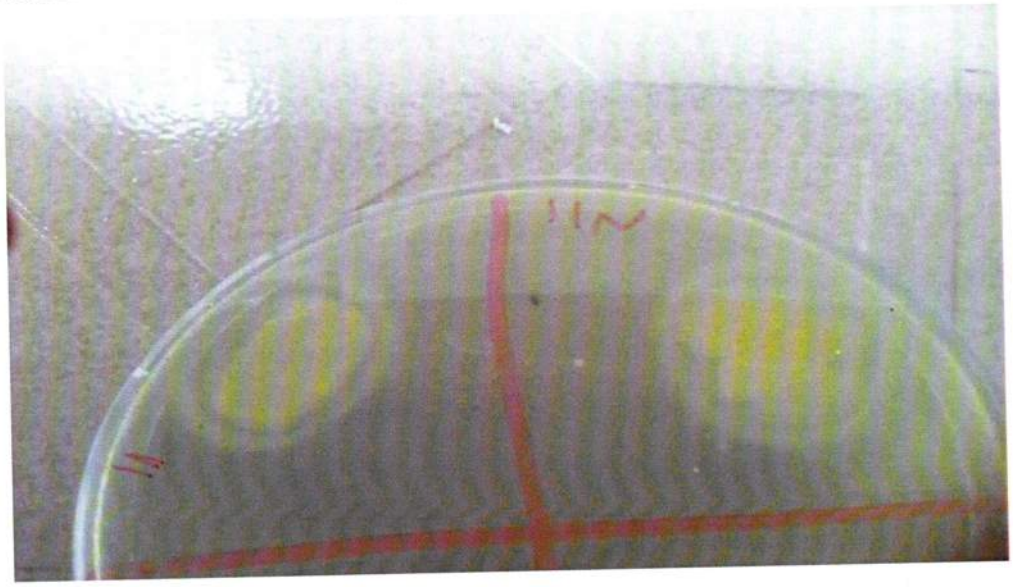

Figure (5):Lipase enzyme activity of *C. acnes* on Spirit Blue agar medium, before treatment (Positive result).

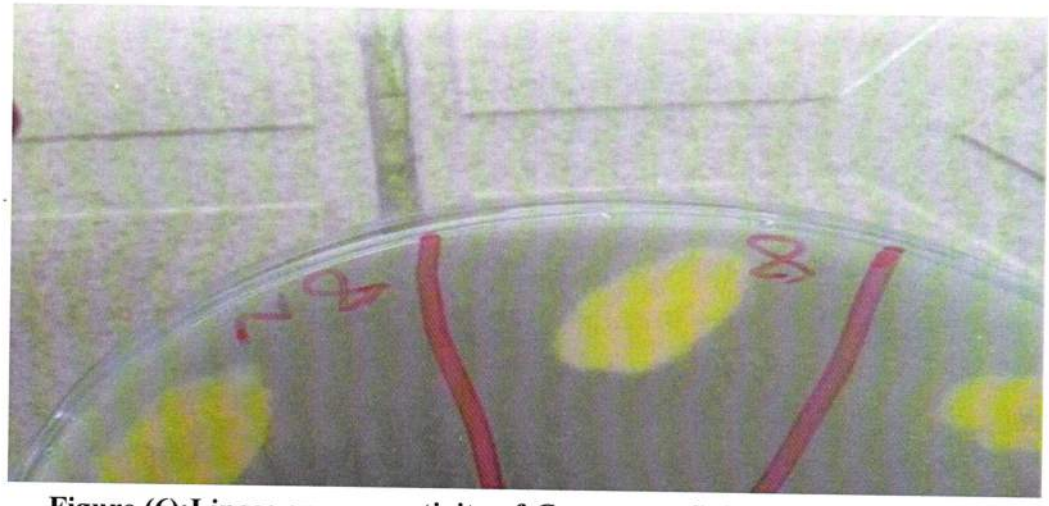

Figure (6):Lipase enzyme activity of *C. acnes* on Spirit Blue agar medium, after treatment (Negative result).

Conclusions and Recommendation:

The *C. acnes*is likely to be a main skin microflora in pilosebaceous unit as it was isolated from all the patients in current study group. Lipase activity of *C. acnes* was determined in all cases isolated from the study group, which was nearly disappeared after therapy. Therefore, Great emphasis should be done on choosing the drug that is used to inhibit or decreases the sebum production and hyperkeratinization activity rather than hypercolonization of *C. acnes*. So, the use of antibiotic as an option in acne treatment is not a good choice, because the *C. acnes* is the predominant part of the skin normal flora, and we must not eradicates this bacterium by using antibiotics. This current study focused on the prevent the optimal environment for *C. acnes* in pilosebaceous follicle that inhibit their virulence factors rather than killing the bacteria as a curing target for acne vulgaris patients.

The better understanding of the role of virulence factors of *C.acnes* other than lipase activity that may allow for new perspective in the treatment of acne. Also, molecular biology combined with pharmaceutical research is necessary to clarify treatment modalities issues.

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