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Assessment of lipase activity for *Cutibacterium acnes* in relation to acne vulgaris disease severity in a group of Iraqi patients

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Abstract:
Background:*The bacterium Cutibacterium acnes can secrete several bacterial products; lipase is the most important of these enzymes in the pathogenesis of acne vulgaris. It metabolizes sebum; targeting an inflammatory response in skin and rolling the disease outcome. The main aim is to assess lipase activity concerning disease severity.*
Materials and Methods:*A cross-sectional study was held in Iraq from November 2018 to June 2019; Where 50 patients suffering from acne vulgaris were enrolled. Skin biopsies were taken from the patients and cultured anaerobically in Thioglycolate broth at 35-37 ^{O}C for 24-48 hours to grow C. acnes. The broth optical density (O.D.) then is estimated to assess the activity of lipase enzyme through assessing the rate of clearance of lipid substrate added to the samples.*
Results:*Anaerobic culture of skin biopsy specimens revealed that 90% of cases were showing positive growth of C. acnes and positive lipase activity. The comparison of O.D. for the Thioglycolate broth measured immediately after the addition of Lipid substrate and that after two hours showed a highly significant statistical difference between the means using paired T-test (P=0.001). A comparison between the mean decrease in O.D. of the mild/moderate cases and severe cases after two hours of lipid substrate addition showed a highly significant statistical difference between the means using unpaired T-test (P=0.008).*
Conclusion: *the bacterial lipase activity is more noticed in severe acne conditions, which reveals the effect of lipase activity on the severity of acne vulgaris.*
Keywords: *Cutibacterium acnes, lipase activity, acne vulgaris, severity*

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I. Introduction

Acne vulgaris, a common skin disease mainly caused by Cutibacterium acnes (previously namedPropionibacterium acnes), is a polymorphic disorder that is characterized by comedones formation1,2.Several interacting factors are enrolled in the pathogenesis of acne vulgaris, such as sebum overproduction which is hormonally determined, follicular hyperkeratosis, microbial flora changes, immunological processes, and inflammation. These factors influence each other and can produce a favorable environment for *C. acnes*3. The bacterium Cutibacterium acnes can secrete many bacterial products like lipase, protease, neuraminidase, hyaluronidase, and lecithinase. The most important in the pathogenesis of acne vulgaris is lipase enzyme which metabolizes sebum; the resulting metabolites have the ability to target an inflammatory response in the skin 4-6. As a result of lipase over-expression, follicular development in acne vulgaris is increased, rolling the disease outcome7.This "Microbial lipase theory" emerged in the last century on the idea that in vivo production of lipase enzyme by *C. acnes* hydrolyzes sebum to produce free fatty acids, which are irritant and comedogenic8. The aim of the current study is to assess the lipase activity for *C. acnes* in relation to the disease severity.

II. Materials and Methods

The current prospective study wascarried out on Salahaddin General Hospital/ Dermatology Consultation Clinic, the study was carried out during the period from the first of November, 2018 to the 30th of June, 2019; Where 50 patients aged 15 -35 years old of both genders suffering from acne vulgaris with different manifestations were enrolled, overcoming the exclusion criteria.

Study Design: Prospective cross-sectional community- based study
Study Location: This was a tertiary care teaching hospital-out clinic based study done in Department of Dermatology and Venereology.

Study Duration: November 2018 to end of June2019.
Sample size: 50 patients.
Sample size calculation: The sample was selected from a target population of 5000 individuals attended the outpatient clinic during the period of study on the basis of single proportion design. The assumption of 95% confidence level was carried out with 10% confidence interval. A drop out of 5% rate was outlined.
Subjects & selection method: Samples were taken from the back of the patients aging 15-35 years of both genders suffering from acne vulgaris with different manifestations attending Salahaddin general hospital/ Dermatology Consultation Clinic from Dec 2018 to the end of June 2019.

Inclusion criteria:
1. Acne vulgaris with different clinical manifestations
2. Either gender
3. Age from 15-35 years
4. Normal lipid profile tests

Exclusion criteria:
1. Abnormal lipid Profile tests
2. Pregnant women
3. Lactating mothers
4. Patients on oral contraceptive pills
5. Patients on antiandrogen
6. Patients on systemic antibiotics
7. Patients on oral retinoid for the last 3 months prior to the study
8. Patients on topical medication within one month prior to the study
9. Patients with chronic diseases (e.g. pancreatitis)
10. Patients with smoking or alcohol intake

Procedure methodology: Samples (i.e. skin punch biopsy specimens) were taken from the back of the patients with acne vulgaris and cultured anaerobically in long butt tubes of Thioglycolate broth at 35-37 ^{O}C for 24-48 hours to grow *C. acne*s.Subculture from the broth media was made (about 0.1-0.2 ml taken from the bottom of long butt tubes using a micropipette to ensure anaerobic condition) on Brucella agar medium supplemented with furazolidone and cultured anaerobically with the aid of strict anaerobic jar at 37 ^{O}C to isolate and identify *C. acnes*, and also on spirit –blue agar with same anaerobic conditions for the detection of lipase activity for *C. acnes*.About 1 ml of lipase-substrate (a combination of tributyrin + polysorbate 80) for bacterial lipase enzyme was added to the Thioglycolate broth culture to allow the bacterial enzyme to act after incubation for 24 hours.Using Spectrophotometer- assay at 450 nm, the broth optical density then is estimated twice (i.e., before culture and after 2 hours from the addition of lipid substrate) to assess the activity of lipase enzyme through assessing the rate of clearance of tributyrin + polysorbate 80 emulsions for all the samples in the current study[9].

A well prepared questionnaire was used to collect information from the target population on the current study, collectingsocio-demographic characteristics (age, sex, nationality, residence, marital status, lifestyle habits as smoking and alcohol intake), and a written informed consent from all the patients enrolled in the study was obtained.All authors declare that the research was conducted in accordance with the Declaration of Helsinki, and under the terms of all relevant local legislation.

Statistical analysis: Statistical analyses were done using GraphPad software. Pearson Chi-square test was used to assess the significance of the association between categorical variables. P Value of ≤ 0.05 was considered as statistically significant.

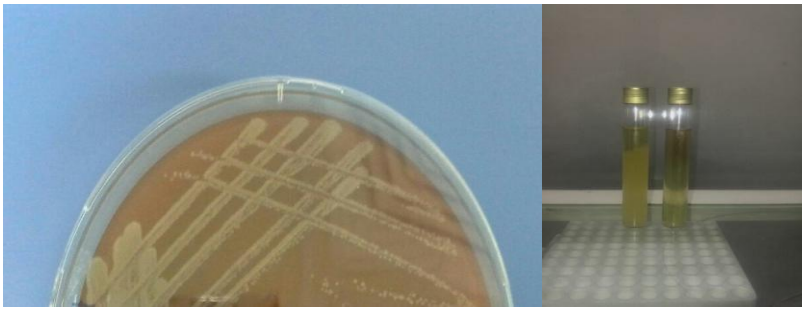
III. Results

Out of 50 patients having acne vulgaris whom are involved in the current study; 30 (60%) of them where females, while the rest 20 (40%) where males. The female to male ratio is (1.5:1). About 72% of these cases were in the teen age group, with the mean age of 18.5 years. The distribution of acne conditions severity is shown in table (1). Mild and moderate cases (i.e. patients presented with papules and/or pustules as the majority of lesions) were presenting about 60% of cases, while the rest 40% were presented with severe manifestations (i.e. nodulocystic majority of lesions).

Table no 1: Distribution of cases according to severity of the condition.

Severity of the lesions	No. of cases	Percentage
Mild / Moderate	30	60 %
Severe	20	40 %
Total	50	100 %

Anaerobic culture of biopsy specimens revealed that 45 cases (90%) were showing positive growth of C. acnes, using the conventional methods for isolation and identification on selective Brucella agar (figure 1), the rest 5 cases (10%) were showing negative growth.



A B

Figure no1: The growth of *C. acnes* on selective Brucella agar (A) and Thioglycolate medium (B).

On Spirit- blue agar, all of the 45 isolates of C. acne (i.e. 100%) were showing positive lipase activity (figure 2). The comparison of optical density (O.D.) of the Thioglycolate broth medium for all isolates measured immediately after the addition of lipid-substrate and that after two hours are shown in table (2). A highly significant statistical difference was noticed between the means using the paired T-test (P=0.001).

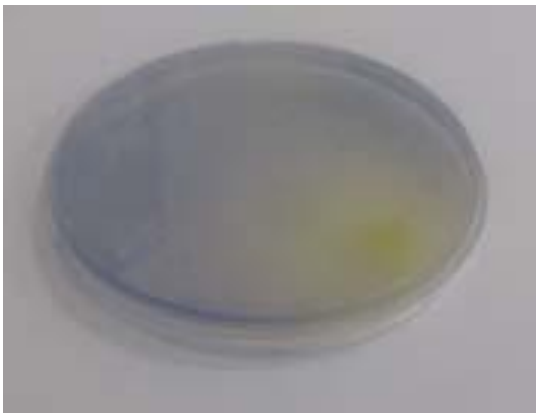


Figure no 2: The growth of *C. acnes* on Spirit-blue agar with lipase activity (i.e., change in color to yellow).

Table no 2: Statistical comparison of O.D. means taken immediately and after two hours using paired T-test (n= 45).

Optical density timing	Mean of O.D.	Standard deviation	Standard error mean	Degree of freedom	P- value
Immediately	0.534	0.229	0.034	88	0.001*
After two hours	0.212	0.034	0.005		

*** Highly Significant**

Table (3) showed a comparison between the mean decrease in O.D. of the mild/moderate cases and severe cases after two hours of lipid-substrate addition. A highly significant statistical difference was noticed between the means using unpaired T-test (P=0.008).

Table no 3: Statistical comparison of the decrease in O.D. means after two hours using unpaired T- test.

Severity of the lesions	Mean decrease in O.D.	Standard deviation	Standard Error mean	Degree of freedom	P- value
Mild/ Moderate (n=25)	0.353	0.176	0.035	43	0.008*
Severe (n=20)	0.534	0.258	0.058		

*** Highly Significant**

IV. Discussion

Cutibacterium acnes produce enzymes such as lipases, proteases, and hyaluronidases, which cause subsequent inflammatory reactions in the surrounding dermis. It seems that the most important factor in inducing severe inflammatory reaction is the free fatty acids formation as a result of C. acnes lipases on sebaceous triglycerides (10,11). Also, the Lipase enzyme may have a crucial role in facilitating bacterial colonization in a nutrient-limited environment- such as human skin. The current study revealed that all of the 45 isolates of C. acne were showing positive lipase activity, which revealed the importance of this enzyme in the pathophysiology of acne condition. Therefore, medications targeting acne have to inhibit C. acnes lipase activity (12).

In the current study, the females were more affected than males, which agreed with epidemiological studies concerning this condition, also, acne vulgaris condition was presented mainly in adolescence, which is attributed to the effect of androgens in this age group (13-15).

The comparison of optical density (O.D.) of the Thioglycolate broth medium for bacterial isolates measured immediately after the addition of lipid substrate and that after two hours showed a highly significant statistical difference (P=0.001), which shows the significant role of lipase activity produced by C. acnes in lipase substrate clearance.

This result is similar to a study conducted by Whiteside and Voss in the USA. They found also that the isolates of C. acnes from patients receiving antibacterial were slightly, but significantly, more actively lipolytic (16).

Kellum et al (17) had reported that the comparison of lipolysis during the growth of their strains had shown more extensive lipolysis by strains from acne than from non-acne sources, and the rates of lipolysis by broth cultures were determined potentiometrically.

The significant decrease in O.D. of the Thioglycolate broth medium inoculated with C. acne strains that were isolated from severe acne condition compared with that of C. acne strains isolated from mild/moderate cases revealed the significant relation between lipase activity and the severity of the diseases. Reports by Salomon et al (18) had shown reduced fatty acid levels in acne. Kanaar (19) had also observed a similar reduction. These findings raise questions concerning the importance of free fatty acids as a determining factor in acne.

V. Conclusion

Bacterial lipase activity is more noticed in severe acne conditions compared with that in mild/moderate acne vulgaris patients, which may reveal the effect of lipase activity on the severity of acne condition.

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