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Effectiveness of *Pseudomonas Aeruginosa* in Destruction of Harmful Lipids in Lipidemia Patients

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Abstract

Pseudomonas aeruginosa is a type of harmful microorganism that belongs to a large group of Gram-negative bacteria, these bacteria can secrete many extracellular enzymes such as microbial lipase and oxidase that act on certain lipids. This study aims to activate certain enzymes of *P. aeruginosa* like lipase and oxidase in order to decrease high plasma triglycerides and cholesterol in lipidemia patients. Serum can also be used due to the fact that lipids are found in both serum and plasma. Five ml. of blood were collected from 100 lipidemia patients at Baghdad Teaching Hospital laboratories in Medical city, *P. aeruginosa* was isolated from medical wastes; cultured on bacterial media, gram stained and biochemically tested *in vitro*. Methods depend on inoculation of bacterial broth containing colonies of *P. aeruginosa* adjusted to McFarland standard turbidity with 50% of blood plasma were added, then cultures were incubated at 37°C for 6 days, activation of bacterial lipase and oxidase were done during the incubation period by addition of certain activators to the broth like glycogen liquid. The results showed that 100% of patients had cholesterol <200 mg/dl and 99% of patients had normal triglyceride level (<200mg/dl) after bacterial inoculation and activation of microbial lipase and oxidase. As conclusion, the high plasma lipids level of patients was decreased four times by *P. aeruginosa* may be due to the activation of microbial lipase and oxidase which could needs to future research to extract them and used them as treatment for these cases.

Keywords: *Pseudomonas aeruginosa*, Cholesterol, Triglycerides, Lipidemia.

الخلاصة

تعتبر الزائفة الزنجارية من الاحياء المجهرية الخطرة والتي ترجع في تصنيفها الى البكتريا التي تأخذ صبغة كرام السالبة، حيث ان هذه البكتريا بإمكانها افراز عدة انزيمات خارج الخلية مثل انزيم اللايبيز والأوكسيديز والتي بدورها تعمل على بعض انواع الدهون. الهدف الرئيسي من هذه الدراسة هو لتحديد الدور الرئيسي للزائفة الزنجارية في تحطيم الدهون الضارة لفئة المرضى الذين يعانون من ارتفاع الدهون في بلازما الدم، كما ويمكن استخدام المصل أيضا وذلك لحقيقة وجود الدهون في كلا من المصل والبلازما لدم الانسان. تم جمع ٥ مل من دم المرضى المراجعين لمختبرات مستشفى بغداد التعليمي في مدينة الطب والذين كانوا يعانون من ارتفاع نسبة الدهون في بلازما الدم وعددهم الكلي ١٠٠ مريض في هذه الدراسة، كما وتم عزل البكتريا الزائفة الزنجارية من الفضلات الطبية والتأكد من تشخيصها مختبرياً بواسطة زراعتها على اوساط زرعية خاصة وعمل صبغة كرام وبعض الفحوصات الكيمياوية. بعد التطعيم في المرق البكتيري لبكتريا الزائفة الزنجارية الذي يحتوي على مستعمرات نقية مضبوطة بواسطة الماكفرلاند القياسي ذو المعيارية ٠,٥ مع خلط نسبة ٥٠٪ من بلازما الدم لمرضى ارتفاع الدهون، وقد تم حضن العينات بحاضنة مختبرية بدرجة حرارة ٣٧ سليزية ولمدة ستة ايام ثم اضافة مواد محفزة ومسرعة لإفراز انزيمي اللايبيز والاوكسيديز من البكتريا كالكليسيرول السائل. اوضحت النتائج ان نسبة الدهون الثلاثية والكوليستيرول بعد التحفيز والتطعيم البكتيري قد قلت الى النسبة الطبيعية اي اقل من ٢٠٠ ملكرام. كما ونستنتج ان نسبة الدهون المرتفعة في بلازما الدم لهؤلاء المرضى قد قلت الى اربعة اضعاف باستخدام هذه البكتريا وربما يعود هذا السبب الى تحفيز البكتريا لإفراز انزيمي اللايبيز والاوكسيديز والتي من الممكن استخدامهما في المستقبل للأبحاث العلمية الخاصة بالاستخلاص واستخدامهما كعلاج لمثل هذه الحالات.

INTRODUCTION

Pseudomonas aeruginosa is a highly contagious organism strictly aerobic bacilli that is widely distributed around the world. Two distinct colored pigments, yellow-fluorescein and pyocyanin, a blue-violet color *in vitro*, are secreted by *P. aeruginosa* spices. [1-3].

Bacterial locomotion is approved by the aid of one flagellum and lives in many hosts, like humans, plants, dusty soil, and water [2][4]. Overdosing

antibiotics may cause the killing of normal flora or modification to resistant strains [4-7]. Lipases degrade cholesterol into glycerol and fatty acids. Lipases are broadly present environmentally, whereas microbial lipases are trading-commercial essentials [8]. Several microorganisms are known to produce lipases that participate in many industrial agents like vegetable oil and waste wafers [9]. Lipase enzymes of bacteria are particularly extracellular enzymes and are specifically affected by many biochemical and physical agents and inorganic sources such as pH and temperature [10]. Regarding enzymes in future, supposed studies will show that they are necessary for many purposes including chemical, medical and commercial uses and treatment [11]. Lipases application may reach digestion studies of large harmful molecules with tumor necrosis factor, enhancing their ability to be effective against cancerous cells. Although, lipase of the stomach is the most stable in acidity, it has proven good in the treatment of intestinal and gastric problems [12][13]. One of the most essential extracellular enzymes secreted by *Pseudomonas* species is cholesterol oxidase, which aids the cholesterol oxidation to produce fatty acid and glycerol as the end product of the reaction [14][15]. Cholesterol Oxidase received much interest regarding its medical uses and application in the lowering of cholesterol in blood & in the breakdown of dietary cholesterol, which then be used in cardiovascular diseases [16][17].

The target of this study is mainly to activate the role of microbial lipase and oxidase of *P. aeruginosa* in destroying high plasma lipids as a trial for future extraction of these enzymes which are used in biotechnologies as a good treatment.

MATERIALS AND METHODS

In the cross-sectional study, 5 ml. of blood was collected from 100 lipidemia patients diagnosed with high levels of cholesterol and triglycerides from the laboratories of Baghdad Teaching Hospital in the Medical City during a period from February 2022 to April 2022, as well as *P. aeruginosa* isolated from surgical waste tools and other contamination sources. All bacteria were diagnosed by growing on several laboratory culture media with further identification tests. Special biochemical kits for the determination of plasma cholesterol and triglyceride levels were used and lipid concentrations were measured by a BTS 350 biosystem chemical analyzer.

All medical waste specimens for *P. aeruginosa* isolation were cultured on MacConkey agar, blood agar for pigmentation (Figure.1a, 1b), brain heart infusion broth (BHI) for long-term saving (Figure 2). Many biochemical tests were used for identification of species like Indole, Methyl- red, Voges- Proskauer test, Simon's Citrate, Triple - Sugar Iron Agar (TSI), oxidase, catalase & lipase activity tests according to Didhiti Agarwal et al, [18]. Blood Agar medium are considered AN ordinary medium for morphological features of bacteria in this study which is characterized by yellow to green pigmented colonies. A differential medium which is MacConkey that is used for the cultivation of Gram-negative bacteria that appear as a flat, non-pigmented colony due to its non-lactose fermenter. Broth media like nutrient or brain-heart broth considered a particular liquid containing medium for growth of particular and very sensitive microbes like certain bacteria and fungi, BHI prepared by mixing 37g of broth in 1 liter of distilled water, then heated to dissolve completely and autoclaved at 121°C for 15 minutes then poured in a special container for further bacterial culture saving as in (Figure 1) [18].

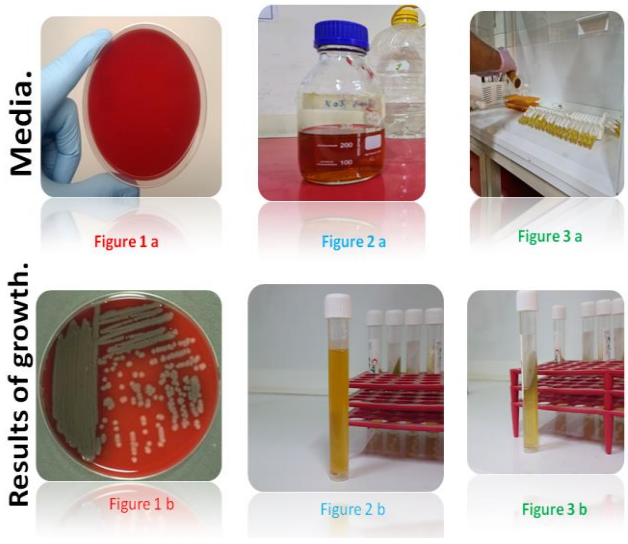

Figure 1. Bacterial different culture media and isolation (1-blood & MacConkey, 2-3 (BHI).

Bacterial broth was injected into plasma containing high lipids by adding 5 ml of the BHI broth containing bacteria at a concentration of 0.5 McFarland turbidity was added with 5 ml of plasma, which contains a high percentage of lipids. Dilution of plasma with bacteria by 50%. This mixture was incubated in the incubator for six days

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at 37 °C. Lipid levels concentration was measured several times via BTS 350 biosystem auto analyzer equipment by using special biochemistry lipid kits (Biosystem Company/Spain). The general purpose for standardizing the turbidity of bacterial broth culture is equal to the capacity of bacterial growth according to 0.5 normality of McFarland standardization with a constant giving $1.5*10^8$ bacterial number. The effectiveness and production of lipase depend on many factors. These factors included temperature, pH, substrate, glycerol, starch and fructose, all these additions increased the production and effectiveness of lipase results [19].

Statistical Analysis

The data of these results were entered into the SPSS version 25 and produced for nominal variables and the Chi-square test.

RESULTS AND DISCUSSION

One hundred lipidemia patients were included in this study concerning the normal level of cholesterol and triglycerides in human plasma (up to 180 mg/dl) and (<200 mg/dl), respectively. This study's results revealed the abnormally increasing level of lipids of triglycerides in about 80% of patients who had TG levels >400 mg/dl with 90% of patients had cholesterol levels between (200-400mg/dl) at highly significant differences as showed in (Table 1).

Table 1. Distribution of 100 study groups according to results of lipids.

Lipids mg/dl	Cholesterol (No%)	Triglyceride (No%)	Total (No%)	*P*-value
<200	10(10%)	8(8%)	18(9%)	* 0.000 HS
200-400	90(90%)	12(12%)	102(51%)	
>400	0(0%)	80(80%)	80(40%)	
Total	100%	100%	100%	

***p-value less than ≤0.01 considered statistically highly significant (HS).**

High activity of oxidase and lipase were activated in bacterial inoculation of *P. aeruginosa* and introduced to the high plasma lipids of patients. The results showed that plasma of lipedema patients returned to normal levels about 100% of patients had cholesterol levels <200 and 99% of patients had triglyceride levels <200 to normal level in plasma at p-value (0.6) as showed in (Table 2).

Table 2. Distribution of 100 study groups after bacterial inoculation.

Lipids mg/dl	Cholesterol (No %)	TG (No %)	Total (No %)	*P*-value
<200	100%	99(99%)	199(99.5%)	* 0.6 NS
200-400	0(0%)	1(1%)	1 (0.5%)	
>400	0(0%)	0(0%)	0(0%)	
Total	100%	100%	100%	

***p-value >0.05 considered statistically non-significant (NS).**

The patients plasma in our study revealed high plasma levels of total lipid with 300 mg/dl cholesterol at a range of (160-300mg/dl) and 800 mg/dl triglycerides at a range of (180-800mg/dl), but after inoculation of 50% bacterial pure colonies of broth controlled with 0.5 McFarland turbidity standard and mixed with 50% patients plasma, then incubated for 6 days at 37°C, the results of lipids were decreased to four times for total cholesterol and triglycerides reaching to a normal range of (40-75 mg/dl) and (45-200mgldl) for cholesterol and triglycerides, respectively, at non statistically significant correlation as showed in (Table 3).

Table 3. Lipids range before & after culture inoculation.

Patients group	Patients Lipids mg/dl		Lipids & broth in 6 days incubation (mg/dl)		*P*-value
	TC.	TG.	TC.	TG.	
1-9	160	180	40	45	* 1.0 NS
10-19	200	320	50	80	
20-29	210	420	52	105	
30-39	220	450	55	113	
40-49	230	500	57	125	
50-59	240	530	60	133	
60-69	250	550	62	138	
70-79	260	570	65	144	
80-89	270	600	67	150	
90-99	280	700	70	175	
100	300	800	75	200	
Range	160-300	180-800	40-75	45-200	

***p-value >0.05 considered statistically non-significant (NS).**

Numerous microbes secrete extracellular enzymes that were utilized previously for medicinal and biological purposes to mitigate the impacts of elevated lipid levels in plasma. Lipases and oxidases are among these enzymes that had been extensively utilized in reducing high cholesterol and triglyceride levels in the blood stream [20].

Cardiac issues and disorders that are caused by artery hyperlipidemia being increased, leading to the use of lipid therapy alongside treatment for

enzymatically related conditions, agree with the study of Saranya et al., 2014 [21].

Jubete et al, (2009) suggest that cholesterol sensors can be beneficial for the food industry, such sensors work by using enzymes that break down cholesterol [22].

The most common lipase-producing bacteria are *Candida*, *Penicillium*, *Pseudomonas* and *Rhizopus* species [23]. Lipases, which are glycoproteins generated extracellularly, are produced by all bacteria. Many microbial lipases are assumed to have non-specific activities, and only a few are heat-stable, with the bulk requiring fatty acids and glycerol as activators [24].

The current study's findings were in agreement with the study of Anil Khatape, whose results concerned in the activation of the bacterial lipase enzyme to destroy lipids was published in India in March 2015 [19]; in addition to the study of Loreena A. Johnson's, whose studies about the breakdown of the milk triglycerides by the bacterial lipase gene activity [25]. These previous studies support the findings of our study by using bacterial broth to degrade high plasma triglycerides following lipase enzyme activation. The current study determines the effectiveness of bacterial lipase and oxidase in the degradation of high plasma lipids in patients by the use of certain activators like starch, sucrose and glycogen to further future biomedical applying as a good effective treatment.

CONCLUSIONS

We can conclude that microbial enzymes such as oxidase and lipase could be effective in reducing blood lipids in lipidemia patients. Thus, it could be useful to conduct future researches to activate the production of these enzymes from bacteria and extract them and used them for treatment of lipidemia patients and heart diseases.

Ethical approval

All participants agreed to provide the investigator with the specimens. The ethics committee of the College of Health and Medical Techniques, Middle Technical University and Ministry of Health, Baghdad Teaching Hospital laboratories in the Medical City approved this work.

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Conflict of Interests: The author affirms that the publication of this paper is not impacted by any conflicts of interest.

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