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The Relationship between *Propionibacterium Acnes* and Free Fatty Acid Levels of the Skin Surface in Acne Vulgaris Patients

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Abstract

For a long time hyperseborrhoea has been considered as a major aetiopathogenetic factor for acne. However, emerging data on alterations of sebum lipid composition in acne patients. Indicate that sebum composition may be more important for the development of acne lesions than the secreted amount. Indeed, bacterial hydrolases convert some of the triglycerides to free fatty acids on the skin surface. Free fatty acids were detected to express proinflammatory and anti-inflammatory properties. The study aims to investigate the relationship between *Propionibacterium acnes* and free fatty acids at the surface skin of acne vulgaris. The study was conducted at a senior high school in Makassar from July to August 2017. The method is analytic observational with cross-sectional design. The samples are acne vulgaris patients aged between 15 to 18 years old in senior high school of the city. Comedones were taken on acne vulgaris lesion from the 28 samples for the examination of *Propionibacterium acnes* and sebum using absorbent paper to examine free fatty acid content. The study indicates no significant correlation between free fatty acid from the skin surface of the patients and the presence of *Propionibacterium acnes*. There is a correlation between *Propionibacterium acnes* and acne vulgaris but insignificant.

Keywords: Acne vulgaris; free fatty acids; *Propionibacterium acnes*.

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1. Introduction

Acne vulgaris is a multifactorial skin disease. The major pathogenetic factors of acne vulgaris have been identified as abnormal ductal keratinization, increased sebum production resulting in seborrhea, an excessive increase of *Propionibacterium acnes* and inflammation. Induction of inflammatory signalling in the pilosebaceous unit is a major component in the process of the initiation of acne lesions [1]. Although acne is not an infectious disease, three major organisms have been isolated from the pilosebaceous ducts of acne patients – *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Malassezia furfur* [2].

The cause of acne is still not fully understood until now. However, there are four major factors leading to the formation of lesions in acne: 1) increased production of sebum by sebaceous glands, in which androgens play an important role, 2) follicular hyperkeratinization, leading to an ultrasound eventually becoming comedones, 3) colonization of the follicle by anaerobic bacteria *Propionibacterium acnes*, 4) inflammatory reaction. Inflammation is further enhanced by rupture of the follicle and followed by the release of lipids, bacteria, and free fatty acids into the dermis [3].

Propionibacterium acnes is a microaerophilic organism in acne lesions. The resulting lipase enzyme can break down diacylglycerol and triglyceride sebum into glycerol and free fatty acids that can trigger proliferative hyperkeratosis of the follicular tube leading to blackheads. Its irritant effect (decreasing pH) gives effect to the breaking of comedones. Protease from *Propionibacteria* allows permeation of follicle contents through the follicle wall and hyaluronidase supports the spread of follicular contents into the dermis. The clinical consequences of this process are the formation of papules, pustules, indurations and abscesses. In addition colonization of *P.acnes* in pilosebaceous follicles is a major factor for inflammatory reactions in acne vulgaris; Therefore, *P.acnes* has been a major target of therapy in acne inflammation [4,5].

Lavker and his colleagues has shown a strong correlation between *P.acnes* density and skin surface lipid count. *P.acnes* may aggravate abnormal keratinization of the follicle. Demonstrations show that free fatty acids can cause comedones in the albino rabbit ear model coupled with evidence showing that 95% of free fatty acids originate from triglyceride hydrolysis of sebaceous glands by *P.acnes* and show that after colonization of *P.acnes* intensive abnormal keratinization occurs [6]. Other evidence supporting the role of free fatty acids in the pathogenesis of acne are: (1) irritating effects of free fatty acids, (2) comedogenic effects of free fatty acids, (3) decrease in free fatty acid levels on the skin during tetracycline therapy. The hypothesis that supports free fatty acids in acne is a hypothesis of microbial pathogenesis in acne [7]. Due to the irritating and comedogenic effects, free fatty acids play a role in various changes in the follicle causing acne lesions [8].

Based on the above background, the researchers are interested in conducting research. The aim of this study is investigate the relationship *Propionibacterium acnes* and free fatty acids at the surface skin of acne vulgaris.

2. Materials and Methods

2.1 Time and Place

Location and time of the study was conducted in the Dermatovenereology Department of Hasanuddin University Hospital, senior high school in Makassar City. The Microbiology Laboratory of Hasanuddin University Hospital, The Chemical Engineering Laboratory of Ujung Pandang State Polytechnic which has held from July to August 2017.

2.2 Design and Variable Research

This research in an observational research using cross sectional design. The research variables consist of independent variable (*Propionibacterium acnes*), dependent variable (*Acne Vulgaris*), and confounding variables (free fatty acid).

2.3 Population and Sample Research

In the Study, the classifications were mild, moderate stage of acne in senior high school in Makassar. The samples of this study were the entire affordable populations that met the inclusion and exclusion criteria.

2.4 The Method

The patients with acne vulgaris in senior high school in Makassar met the inclusion and exclusion criteria for anamnesis, physical examination, and were asked to be involved in the study after signing informed consent. Physical examination is performed to confirm the diagnosis of mild and moderate acne vulgaris. Furthermore, PCR Examination and Gas Chromatographic Examination. The data obtained will then be analyzed and reported as research results.

2.5 Data Analysis

Data in this research will be processed with the help of computer using SPSS version 18. Data processing is analyzed with T.Test and Chi square. All results of the analysis will be presented in tabular form with annotations.

3. Results

The study was conducted in Makassar, South Sulawesi by taking samples of 28 acne vulgaris patients who met inclusion criteria, consisting of 14 subjects with mild degrees of acne vulgaris and 14 subjects with moderate degrees of acne vulgaris, obtained from senior high school in Makassar City. Examination is done with attention to clinical manifestations. Sampling of specimens in the form of sebum with absorbent paper media for the examination of free fatty acid of the skin surface with gas chromatography at Chemical Engineering Laboratory of Ujung Pandang State Polytechnic and taking specimen of comedones lesion to know the presence or absence of *Propionibacterium acnes* by PCR examination.

Based on the characteristics of the samples, 28 samples were obtained un this study and the male sample was peoples (71,43%) and women was 8 peoples (28,57%). The 15-16 age group was the highest frequency group

with 22 peoples (78,57%), while the lowest was 6 samples in the 17-18 years age group (21,43%). Based on family history, 17 peoples (60,71%) have family history and 11 persons (39,29%) without family history (Table 1).

Table 1:The characteristics of Acne vulgaris patients in Study

Category	Frequency (n)	Percentage (%)
Sex		
Male	20	71,43
Female	8	28,57
Age		
15-16 y.o	22	78,57
17-18 y.o	6	21,43
Table 1. Continued		
Family history		
Yes	17	60,71
None	11	39,29
Food		
Milk	5	17,86
Chocolate	3	10,71
Oily food	20	71,43
High Sugar food	2	7,14
Total	28	100

The result of PCR *Propionibacterium acnes* examination from 28 people that PCR was not found in *Propionibacterium acnes* on mild degrees of acne vulgaris, whereas in moderate acne vulgaris the degree was obtained with 3 from 14 subjects (21.4%) positive subjects found *Propionibacterium acnes* while negative results were found in all subjects with moderate acne vulgaris. Positive PCR results in AD1, NR4, NR14. Where appropriate with positive control values and tire target was 344 bp (Figure 1 and Table 2).

Table 2: Results of PCR *Propionibacterium acnes* Examination on Group of Mild and Moderate Acne Vulgaris

PCR	mild (n=14)	moderate (n = 14)	Total
Positif	0 (0%)	3 (21,4%)	3 (10,7%)
Negatif	14 (100%)	11 (78,6%)	25 (89,3%)

Table 3 shows the relationship between free fatty acid of the skin surface of acne vulgaris patient with PCR *Propionibacterium acnes* but not significant, $p = 0.538$ ($p > 0.05$). The correlation coefficients of *P. acnes* counts and the amount FFA were negative.

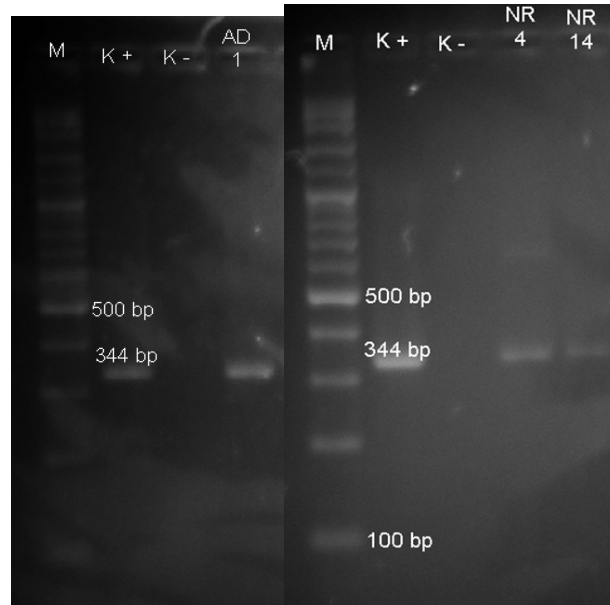


Figure 1: Results of PCR *Propionibacterium acnes*

Table 3: Relationship between PCR *Propionibacterium acnes* with free fatty acid of the skin surface of acne vulgaris patient

Variable	Free Fatty Acid	
	Correlation coefficient	p
PCR <i>P. acnes</i>	-0,122	0,538

4. Discussion

The characteristics of the research sample were 28 subjects of mild and moderate acne vulgaris patients who fulfilled the inclusion criteria obtained age distribution of 15-17 years as many as 27 people, 18 years old as much as 1 person. All age groups may be affected, but acne is a more common disorder in adolescence. Early acne lesions are seen at 8-9 years of age and approximately 50-60% are present in adolescents [3]. The prevalence of 80-85% acne vulgaris in adolescents with peak incidence is 15-18 years, 12% in women aged > 25 years and 3% at 35-44 years. The prevalence of acne vulgaris 15-25 years about 58%.

Family history with acne, especially in fathers and mothers, increases the risk of acne in their children. Several studies have shown that genetic factors play a role in the susceptibility of acne. Research in Germany shows that 45% of acne occurs in boys who have a history of one or both parents suffering from acne and 8% with no history of acne in the elderly [9]. And based on epidemiological studies conducted in schools in France found among 913 adolescents aged 11-18 years with a history of acne in the father obtained 16% results in the acne group compared to 8% in the group without acne lesions. Meanwhile, a history of maternal acne was also

studied and 25% in the acne group and 14% in the group without acne lesions. In the history of acne studied in siblings, there was a 68% gain in the acne group and 57% in the group without acne lesions. In addition, a history of acne in the father or mother is often associated with severe acne events or acne does not respond to treatment [10].

Patients who have the habit of eating oily food as many as 20 people, who have a habit of drinking milk as many as 5 people, who have a habit to eat sugar-sweet foods as much as 2 people and 3 people chocolate. Research Astuti found that the most influential foods on the incidence of acne vulgaris are nuts and fried foods. A previous study reported that foods with high glycemic index can affect the development and severity of acne vulgaris. The effect of food on the occurrence of acne vulgaris is still a debate of experts. However, most patients still argue that food as a cause or factor exacerbates acne vulgaris [11]. Various studies of chocolate on the appearance of acne and concluded that there is no evidence that chocolate affects the formation of acne [12].

The results of PCR sampling of comedones of acne vulgaris patients was show 3 subjects (21.4%) of moderate acne vulgaris patient were found to be positive, while the subjects of mild acne vulgaris were not found to be positive. *Propionibacterium acnes* is usually found as a normal flora of the pilosebaceous glands, gram-positive and anaerobic bacteria. *Propionibacterium acnes* is high in areas rich in sebaceous glands such as the face and head [13]. *Propionibacterium acnes* develops within the pilosebaceous follicle and below to the skin surface together with sebum and *Propionibacterium acnes* has been identified to have high concentrations in microcomedones [14, 4].

Free fatty acids on human skin surface lipids play an indirect role in the pathogenesis of acne vulgaris. Evidence supporting the role of free fatty acids in the pathogenesis of acne are: 1) irritating effects of free fatty acids, 2) comedogenic effects of free fatty acids, 3) reduction of free fatty acid levels on the skin during tetracycline therapy. The hypothesis that supports free fatty acids in acne is a hypothesis of microbial pathogenesis in acne [6]. These lipids are thought to be involved in ductal hypercornification, play a role in bacterial growth, induce an inflammatory response, and are comedogenic because free fatty acids are condensed [9,15].

Marpels and his colleagues shows the role of enzyme lipase *Propionibacterium acnes* in producing free fatty acid by converting triglycerides to free fatty acids and Ingram and his colleagues showed purified *Propionibacterium acnes* lipase will hydrolyze a number of triglycerides and show that *Propionibacterium acnes* in the sebaceous follicle derive nutrients from the degradation of sebum triglycerides to produce free fatty acids through the intermediate lipase work [16]. However, in this research showed there was a relationship between PCR *Propionibacterium acnes* with free fatty acid of the skin surface but not significant.

Free fatty acids can be found on the surface of human skin and are a major component in human sebum. Free fatty acids are produced by the hydrolysis of the sebum triglycerides secreted from the sebaceous glands, with lipases secreted by commensal bacterial flora such as *P. acnes* and *Staphylococcus epidermidis* [17] and from Numata's study and his colleagues which detects *Malassezia* not only samples obtained from the surface of the skin (by swab method), but also samples obtained from inflammatory lesions (via comedones extraction). These results suggest the possibility that *Malassezia* is associated with acne vulgaris. They consider *Malassezia* spp.

may be associated with acne inflammation in some acne patients, because these microorganisms are lipophilic and have lipase activity such as *P. acnes*, and are associated with inflammatory disorders (eg, *Malassezia folliculitis*) [18].

5. Conclusion

The authors concluded that there was no significant relationship between free surface fatty acids of skin of acne vulgaris patient in the presence of *Propionibacterium acnes*. The study indicates no significant correlation between free fatty acid from the skin surface of the patients and the presence of *Propionibacterium acnes*. There is a correlation between *Propionibacterium acnes* and acne vulgaris but insignificant. Researchers suggest that research on the relationship of free fatty acid content with examination of other bacteria suspected causes of acne vulgaris such as *Staphylococcus epidermidis*.

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Competing Interest

The authors declare that they have no competing interests.

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