

The association between *Staphylococcus epidermidis* and palmitic acid level in patients with acne vulgaris

Associação entre *Staphylococcus epidermidis* e o nível de ácido palmítico em pacientes com acne vulgar

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ABSTRACT

Introduction: Acne vulgaris is an inflammatory disorder of the pilosebaceous gland. Palmitic acid is one of the major types of free fatty acid and may play a role in acne pathogenesis. In addition, recent studies suggested that *Staphylococcus epidermidis* might be involved in acne.

Objective: To explore the association between *Staphylococcus epidermidis* and palmitic acid in acne vulgaris.

Methods: Forty-three high school students at an urban area in South Sulawesi, Indonesia, were included. The palmitic acid level was measured using gas chromatography and comedone was cultured to detect the microbiota profile. Mann-Whitney test was used to analyze the median palmitic level difference between groups with different acne vulgaris severity.

Results: Fourteen patients (32.6%) had mild acne vulgaris, while 14 and 15 patients had moderate and severe acne vulgaris, respectively. The severe and moderate group showed significantly higher palmitic acid level compared with the mild group. Subgroup analysis of patients with moderate and severe acne vulgaris positive for *S. epidermidis* showed a significantly higher palmitic acid level compared with the mild group.

Conclusions: This result suggests that *S. epidermidis* may be associated with the palmitic acid level and may contribute to acne pathogenesis.

Keywords: Acne vulgaris; *Staphylococcus epidermidis*; Palmitic acid

RESUMO

Introdução: A acne vulgar é um distúrbio inflamatório da glândula pilosebácea. O ácido palmítico é um dos principais tipos de ácidos graxos livres e pode desempenhar um papel na patogênese da acne. Além disso, estudos recentes sugeriram que o *Staphylococcus epidermidis* pode estar envolvido na acne.

Objetivo: Explorar a associação entre a *Staphylococcus epidermidis* e o ácido palmítico na acne vulgar.

Métodos: 43 estudantes do ensino médio de uma área urbana do sul de Sulawesi, na Indonésia, foram incluídos. O nível de ácido palmítico foi medido utilizando cromatografia gasosa e os comedões foram cultivados para detectar o perfil do microbioma. O teste de Mann-Whitney foi utilizado para analisar a diferença do nível palmítico médio entre os grupos com diferentes graus de gravidade da acne vulgar.

Resultados: 14 pacientes (32,6%) apresentavam acne vulgar leve, enquanto 14 e 15 pacientes apresentavam acne vulgar moderada e grave, respectivamente. O grupo grave e moderado apresentou nível de ácido palmítico significativamente maior em comparação ao grupo leve. A análise de subgrupo de pacientes com acne vulgar moderada e grave, positiva para *S. epidermidis*, mostrou um nível significativamente maior de ácido palmítico comparado ao grupo leve.

Conclusões: Esses resultados sugerem que *S. epidermidis* pode estar associado ao nível de ácido palmítico e pode contribuir na patogênese da acne.

Palavras-Chave: Acne vulgar; *Staphylococcus epidermidis*; Ácido palmítico

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Authors:

Farida Tabri¹

¹ Cosmetic Division, Department of Dermatology and Venereology, Faculty of Medicine, Hasanuddin University

Correspondence:

Farida Tabri

Department of Dermatology and Venereology, Faculty of Medicine, Hasanuddin University.

Jl. Perintis Kemerdekaan KM. 11

Tamalanrea, Makassar, South Sulawesi, Indonesia.

Email: farida.tabri.dv@gmail.com

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INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous glands where lesions can be non-inflammatory (open and closed comedones) or inflammatory (papules and pustules).^{1,2} Acne vulgaris mostly occurs in adolescence to young adulthood and can cause post-inflammatory hyperpigmentation and scarring. A study conducted by Bhate *et al* showed that acne can be found in about 20% of young adults.³ In addition, acne has a high persistence in which 43% of those over 30 years still have acne. Acne also has a strong genetic predisposition in which 80% of acne cases can be inherited to close relatives.^{3,4}

The human skin hosts a diverse group of microorganisms in which *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* constitute 60% of all bacterial species.⁵ However, there are only limited studies examining the entire microbiota population in patients with acne and their role in acne pathophysiology. Despite the widely accepted view of the role of *Propionibacterium acnes* (*P. acnes*) in triggering acne, recent studies have suggested that *Staphylococcus epidermidis* (*S. epidermidis*) may also be involved in acne pathogenesis. Pathak *et al.*⁶ showed that the microbial load of *S. epidermidis* increases in acne lesions compared with control. This result is in line with a recent study where *S. epidermidis* was found in a more abundant manner compared to *P. acne*.⁷, suggesting the possible role of *S. epidermidis* in acne pathogenesis.

Besides microbiome, free fatty acid (FFA) has also been shown to be involved in the development of acne as increased level of FFA will lead to neutrophils influx through chemotaxis process.⁸ A high-fat diet has been shown to induce or aggravate acne vulgaris lesions.⁹ Of all FFA types, palmitic acid and its derivatives have been shown to be the most abundant type.¹⁰⁻¹² Palmitic acid may stimulate the release of various proinflammatory cytokines and contribute to pilosebaceous duct hyperkeratinization and acne inflammation.¹³

To the best of our knowledge, no study has observed the association between *S. epidermidis* and palmitic acid and their association with acne vulgaris. The aim of this study is to investigate the microbiome profile, especially *S. epidermidis*, in patients with acne vulgaris and to investigate its association with palmitic acid level in inducing acne vulgaris lesions.

METHODS

This cross-sectional study was conducted in a high school in Makassar, South Sulawesi, Indonesia, from July to August 2017. The subjects were given explanation about the study and those who agreed were asked to sign an informed consent form (Ref. Number; 145/H4.8.4.5.31/PP36-KOMETIK/2017 from Hasanuddin University Ethics Committee). Subjects who did not take retinoids, antibiotics, anti-inflammatory drugs, and other anti-microbial products, such as antifungal soap and shampoo, in the last one month were assessed for acne severity with the Lehman Criteria and classified into mild, moderate, or severe acne vulgaris.¹⁴ They were also given questionnaires to record family history of acne and dietary consumption.

Sebum was extracted from the forehead, nasolabial,

cheek, and chin by an absorbent paper using acetone and diethyl ether with a ratio of 1:1 and then methylated using 0.2 M phenyltrimethylamine hydroxide solution in methanol. Gas chromatography was used to examine the product. The standard reference used was Supelco® 37 component FAME Mix. The standard concentration used for palmitic acid was 601 ppm injected into the gas chromatograph. Analysis was performed on a GC-MS QP 2010 Ultra Shimadzu Autosampler with a splitless injector. Separations were achieved using SH-Rxi-5Sil MS capillary column (30m x 0.25mm). Helium was used as the carrier gas at flow rates of 1.99mL/min and a split less ratio of 1:10. The injector temperature was 250°C. The oven temperature was programmed at 140°C for a hold of 10 minutes and increased to 250°C with a flow of 7°C/min. It was further held for 10 minutes, resulting in a total analysis time of 35.71 minutes. MS spectra were obtained at range width m/z 40-500m/z, ion source and interface temperature 210°C and 255°C, respectively, and solvent cut time 3 minutes.

Sample from comedonal lesions was taken using a sterile comedone extractor and cultured in blood agar which was incubated at 37°C for 48-72 hours. The growing colonies were observed under the microscope with gram staining. Each colony was made into a suspension equivalent to McFarland 0.5. The gram-positive and the gram-negative suspensions were taken 145µl and 280µl, respectively, and added into reaction tubes containing 3 ml saline solution. The gram-positive suspension was put into GPcard and AST GP 67 cards and the gram-negative suspension was put into GNcard dan ASTN 100 cards before final analysis using Vitek®.

Data analysis was done by using Statistical Package for Social Sciences (SPSS) 18.0 for Windows (SPSS Inc. Chicago, IL, USA). Mann-Whitney test was used to analyze the median palmitic level difference between groups with different acne vulgaris severity. A p-value <0.05 was considered significant.

RESULTS

The demographic data in Table 1 shows that of 43 participants, most participants (33 patients, 76.7%) were men and adolescent (15.77 ± 0.84 years). There were 25 participants with a positive family history of acne vulgaris (58.1%). As for the disease duration, 34.8% of the participants (15 subjects) have been having acne for 2 years and 10 subjects (23.3%) had had acne for 1 year and 3 years, respectively. Most of the patients (31 subjects, 72.1%) consumed fatty foods. The population showed almost uniform acne severity distribution: 14 patients were diagnosed with mild acne, 14 patients with moderate, and 15 patients with severe acne.

Figure 1 shows the concentration of palmitic acid in severe acne (median = 30,400 ppm) was significantly higher than that in the mild acne (30,400ppm vs 12,746ppm). The palmitic acid level in moderate acne was also found to be significantly higher than in the mild acne ($p < 0.05$). However, no difference in palmitic acid level was found between patients with moderate and severe acne.

Table 2 shows the frequency of the microorganism found in the participants. It was shown that *S. epidermidis* was the most common microorganism cultured (32 subjects, 74.41%) and while non-*S. epidermidis* organisms were isolated in 11 cases (25.59%). The isolated non-*S. epidermidis* microorganisms were *Staphylococcus hominis* (4 cases), *Staphylococcus warneri* (2 cases) *Staphylococcus xylosus* (1 case), *Staphylococcus aureus* (1 case), *Staphylococcus haemolyticus* (1 case), *Staphylococcus capitis* (1 case), *Lactobacillus plantarum* (1 case).

Data in Figure 2 shows that there was no association between *S. epidermidis* and palmitic acid level. However, the data in Figure 3 on subgroup analysis of patients with positive *S. epidermidis* culture showed that palmitic acid level in subjects with moderate acne vulgaris was significantly higher compared with mild acne vulgaris ($p=0.006$). Furthermore, subjects with severe acne vulgaris were also found to have higher palmitic acid level compared with those with mild acne with a borderline significance level ($p=0.064$).

DISCUSSION

Data from our study showed that higher palmitic acid level indicated a greater degree of acne severity. Palmitic acid levels in the facial skin of patients with severe and moderate acne vulgaris were found to be significantly higher than those with a mild degree of acne vulgaris. Several studies have suggested the role of palmitic acid in inducing pro-inflammatory

TABLE 1: Basic characteristics of the population

Category	Frequency (n)	Percentage
Gender		
Man	33	76,7
Women	10	23,3
Age (average,SD)	15,77 ± 0,84 anos	
Family History		
Present	25	58,1
Absent	18	41,9
Food		
Milk	7	16,3
Chocolate	3	7,0
Fatty foods	31	72,1
High Sugar Food	2	4,7
Acne Severity Degree		
Light	14	32,6
Medium	14	32,6
Weight	15	34,9
FFA level ppm (median, IQR)	24,358 (12,946-39,838)	
PCR to P. acnes		
Positive (+)	5	11,6
Negative (-)	38	88,4

Fonte: Tabri F, et al. 2018⁵

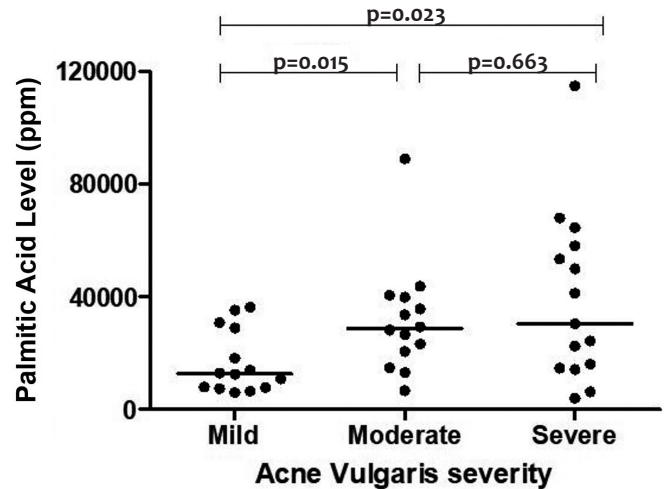


FIGURE 1: Comparison of palmitic acid levels between different acne groups

Source: Tabri F, et al. 2018⁵

TABLE 2: Frequency of microorganisms found in participants

Etiology	Frequency (n)	Percentage (%)
<i>Staphylococcus epidermidis</i>	32	74.41
Non- <i>Staphylococcus epidermidis</i>	11	25,59%
<i>Staphylococcus hominis</i>	4	
<i>Staphylococcus warneri</i>	2	
<i>Staphylococcus xylosus</i>	1	
<i>Staphylococcus aureus</i>	1	
<i>Staphylococcus haemolyticus</i>	1	
<i>Staphylococcus capitis</i>	1	
<i>Lactobacillus plantarum</i>	1	
Total	43	100

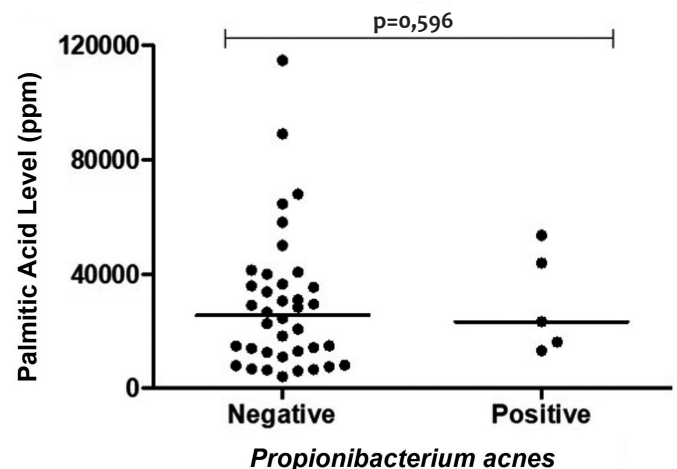


FIGURE 2: Association between palmitic acid and *P. acnes* levels, identified by PCR

Source: Tabri F, et al. 2018⁵

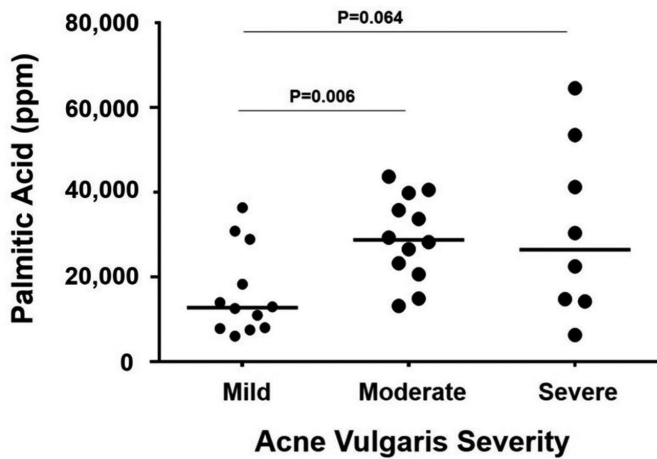


FIGURE 3: Analysis of the association between acne vulgaris and palmitic acid in the subgroups

cytokines, which contributes to inflammation and pilosebaceous duct hyperkeratinization.^{13,15} Palmitic acid was shown to inhibit hydrogen peroxide which caused proinflammatory mediators to pass more easily to the dermis and aggravate inflammation.¹⁵

Data in table 2 showed that *Staphylococcus epidermidis* was the most common microorganism found in the subjects. Interestingly, there was no *P. acne* obtained through culture, despite the classical view that *P. acne* induces acne. Dreno *et al.*⁷ found a similar result, where *S. epidermidis* was the most abundant bacteria found in patients with acne vulgaris. They also observed that *P. acne* only constitutes less than 2% of the whole population. In contrast, other studies showed that in normal population, *P. acne* may represent up to 30% of the whole facial microbiota.^{16,17} One possible explanation for this finding is that the aerobic and facultative anaerobic *S. epidermidis* mediates fermentation process which inhibits the growth of the anaerobic *P. acne*.¹⁸

Although the data in figure 2 shows that *S. epidermidis* was not associated with palmitic acid level, subgroup analysis of patients with positive *S. epidermidis* culture interestingly showed that the palmitic acid level in patients with moderate acne vulgaris to be significantly higher compared with those with mild disease ($p=0.006$). Those with severe acne were also found to exhibit higher palmitic acid level compared with those presenting mild acne with borderline significance level ($p=0.064$). Studies have suggested the possible role of palmitic acid inducing acne vulgaris through the activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway, triggering the activation of toll-like receptor 2 (TLR2), and pilosebaceous

duct hyperkeratinization,^{13,19,20} In addition, the expression of NLRP3 and inflammasome-mediated IL-1 β production are also upregulated which further perpetuates the inflammation process.²¹ Taken together, this result suggests that *S. epidermidis* may be associated with the level of palmitic acid level and hence be involved in the pathogenesis of acne vulgaris. Further biomolecular studies are needed to confirm this hypothesis.

Recent literature showed that *S. epidermidis* may be involved in the pathogenesis of acne vulgaris.²² A study by Bialtecka *et al.* showed that *S. epidermidis* induces inflammation in the form of Nitric Oxide production within the infected tissue. They also induce macrophage activation, as shown by the increase of TNF-alpha production and IL-12 production after incubation with *S. epidermidis*.²³ This topic remains contentious, however, as *Staphylococcus* was shown to activate TLR2 and induce miR-143 in the keratinocytes that target 3' UTR of TLR2 and decrease the stability of TLR2 mRNA and TLR2 protein; resulting in the inhibition of *P. acne* proinflammatory cytokines.²⁴ Also, a study has suggested that fatty acid composition did not associate with the presence of acne, although they might differ in accordance with the amount of triglyceride.²⁶ Furthermore, *S. epidermidis* is able to form biofilm by secreting the exopolysaccharide intercellular adhesion, which supports an anaerobic condition for *P. acne* to flourish.^{27,28} With the above-mentioned data, whether skin microbiome provides a protective or pathogenic effect remains debatable.²⁹

To the best of our knowledge, this is the first study to explore the association between *S. epidermidis* and palmitic acid level and their effect in acne vulgaris. This is an initial study conducted to assess the prevalence of acne vulgaris in the adolescent population of an urban area and its association with palmitic acid levels patients with acne vulgaris. However, as a preliminary study, the sample size is limited and hence some subgroup analysis could be conducted. This study also only assessed one type of free fatty acid. Squalene and its peroxide, for example, has been thought to contribute to inducing inflammation and comedone formation.³⁰ Futures larger scale studies examining more than one type of free fatty acid need to be done. Nevertheless, this study provided important data and thus delivered a foundation for future studies in this field.

The result of this study shows that *S. epidermidis* is the most common microorganism cultured from the participants. In addition, *S. epidermidis* may be associated with higher palmitic acid levels which may contribute to the development of acne vulgaris. Further studies on the underlying mechanism of *S. epidermidis* in inducing palmitic acid level and acne vulgaris need to be conducted to confirm this hypothesis. ●

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DECLARATION OF PARTICIPATION:

Farida Tabri |  ORCID 0000-0003-4913-0909

Design and planning of the study, data collection, analysis and interpretation, preparation and writing of the manuscript, critical review of the manuscript. Approval of the final version of the manuscript.