Original Articles

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Profile of free fatty acid in patients with acne vulgaris

Perfil de ácidos graxos livres em pacientes com acne vulgar

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ABSTRACT

Introduction: Acne vulgaris is an inflammatory disorder that affects the pilocebaceous gland with high prevalence among young adults. Studies have suggested that FFA may influence acne vulgaris. However, the pathogenesis of acne is not yet fully known.
Objective: To analyze the correlation between free fatty acid and acne vulgaris severity level.
Methods: Forty-three female high school students with mild, moderate, and severe acne were included in this study. Free fatty acid level, represented by palmitic acid level, was measured using gas chromatography and PCR examination was conducted to detect Propionibacterium acnes. 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 as significant.
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 acne group showed significantly higher palmitic acid level compared to the mild acne group (p<0.05). The level of palmitic acid was not associated with the presence of P. acnes.
Conclusions: Increased palmitic acid level was found to be associated with acne severity. Thus, FFA levels may be used as a marker to determine acne vulgaris severity
Keywords: Acne vulgaris; Fatty acids, nonesterified; Polymerase chain reaction; Propionibacterium acnes

RESUMO

Introdução: *A acne vulgar é um distúrbio inflamatório que afeta a unidade pilossebácea, apresentando alta prevalência entre adultos jovens. Estudos sugerem que os ácidos graxos livres (AGL) podem influencia-la, contudo, sua patogênese ainda não é totalmente conhecida.*
Objetivo: *Analisar a correlação entre o nível de ácidos graxos livres e a gravidade da acne vulgar.*
Métodos: *Quarenta e três alunas de ensino médio portadoras de acne leve, moderada e grave foram incluídas neste estudo. O nível de ácidos graxos livres, representado pelo nível de ácido palmítico, foi medido por cromatografia gasosa enquanto a detecção do Propionibacterium acnes foi realizada através do PCR. O teste de Mann-Whitney foi utilizado para analisar a mediana da diferença do nível de ácido palmítico entre os grupos com diferentes graus de severidade da acne vulgar. Os resultados foram considerados significativos para valores de p <0,05.*
Resultados: *Catorze pacientes (32,6%) apresentaram acne vulgar leve, enquanto 14 e 15 pacientes apresentaram acne vulgar moderada e grave, respectivamente. Os grupos com acne grave e moderada apresentaram um nível de ácido palmítico significativamente maior quando comparado ao grupo com acne leve (p <0,05). O nível de ácido palmítico não foi associado à presença de P. acnes.*
Conclusões: *O aumento do nível de ácido palmítico mostrou-se associado à gravidade da acne. Assim, os níveis de AGL podem ser usados como marcadores para determinar a gravidade da acne vulgar.*
Palavras-Chave: *Ácidos graxos não esterificados, Acne vulgar, Propionibacterium acnes, Reação em cadeia da polimerase*

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous units[1]; lesions may be non-inflammatory (open and closed comedones) or inflammatory (papules and pustules).[2] Acne vulgaris occurs mainly in adolescence and may cause hyperpigmentation and the formation of postinflammatory scars.[3] A study by Bhate et al. showed that acne can be found in roughly 20% of young adults. In addition, acne has high persistence, and 43% of people over 30 years of age still have acne. Acne also has a strong genetic predisposition, and 80% of cases may have been inherited from close relatives.[3]

The pathogenesis of acne is still not fully understood. However, four mechanisms (follicular hyperkeratinization, colonization by *Propionibacterium acnes* (*P. acnes*), sebum production and complex inflammatory mechanisms involving the innate and adaptive immune system) have been widely accepted as the underlying processes. *P. acnes* is thought to play an important role in the pathogenesis of acne, as it causes tissue damage through the release of various enzymes, including lipase, which breaks down triglycerides into glycerol and free fatty acids (FFA), leading to an influx of neutrophils through chemotaxis.[5] A study showed that FFA induce the growth of *P. acnes* and abnormal follicular keratinization.[6] It is believed that FFA interfere in the dynamics of intracellular calcium in follicular keratinocytes and in the epidermal intercellular lipid bilayer.[7] Among all types of FFA, palmitic acid is the most abundant.[8] It has been demonstrated that palmitic acid can stimulate the release of several proinflammatory cytokines, contributing to the hyperkeratinization of the pilosebaceous duct and inflammation in acne.[9]

Interestingly, another study by Desboies et al., Showed that FFA have the ability to prevent bacterial fixation in the skin. The mechanism of action of these fatty acids usually occurs in cell membranes, through the active transport system of electrons and oxidative phosphorylation. Free fatty acids also inhibit enzymatic activity, nutrient uptake, peroxidase formation, and auto-oxidation. Free fatty acids also have the potential to assist in the reduction of the severity of acne vulgaris.[10]

These conflicting data stimulate further research on the role of FFA in the pathogenesis of acne. Thus, the present study was aimed at evaluating the association between FFA, the severity of acne and the presence of *P. acnes*, with palmitic acid as the measured component in the facial skin.

METHODS

This cross-sectional study was carried out at a high school in an urban area (Makassar City, Indonesia) between July and August 2017. After receiving an explanation about the study, the volunteers who agreed were asked to sign a Free and Informed Consent Form (Ref. Number; 145 / H4.8.4.5.31 / PP36-KOMETIK / 2017, Hasanuddin University Ethics Committee). Those who had not used retinoids, antibiotics or anti-inflammatories in the previous month were assessed for acne severity according to the Lehman criteria, having been classified into bearers of mild, moderate or severe acne vulgaris.[11] These participants also received questionnaires aimed at collecting family history of acne and dietary consumption.

Sebum was harvested using an absorbent paper moistened with 1:1 acetone and diethyl ether, being subsequently methylated with 0.2M phenyl trimethylamine hydroxide solution in methanol. Gas chromatography was used to examine the product. The standard reference used was the FAME Mix component of Supelco® 37. The standard concentration used for palmitic acid was 601ppm, injected into the gas chromatograph. The analysis was performed using a GC-MS QP 2010 Ultra Shimadzu Autosampler, with a splitless injector. Separations were performed using SH-Rxi-5Sil MS capillary column (30m x 0.25mm). Helium gas was used as the carrier gas at flow rates of 1.99 mL/min and a splitless ratio of 1:10. The temperature of the injector was set at 2,500 °C. The oven temperature was programmed to stay constant at 1,400 °C for 10 minutes, being subsequently increased to 2,500 °C, with a flow rate of 7 °C / min. This temperature was maintained for additional 10 minutes, resulting in a total analysis time of 35.71 minutes. Mass spectrometry was obtained in a range corresponding to 40-500 m/z, with the temperatures of the ion source and the interface at 2,100 °C and 2,550 °C, respectively, and solvent cut-off time of 3 minutes. A sample of comedo lesions was analyzed for the presence of *P. acnes* using PCR (Biorad®, California, USA) according to the following primer sequence: forward (PR 264): 5-GCA GGC AGA GTT TGA CAT CC -3, reverse (PPA.R): 5-ATG TTG AGG GCG GTG ACG TT-3, and target ban 344 bp.

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The Mann-Whitney test was used to analyze the difference in median palmitic acid levels between groups with different degrees of severity of acne vulgaris. Differences were considered significant for $p < 0.05$.

RESULTS

Table 1 shows the demographic data and basic characteristics of the study population. A total of 43 individuals were included, with most being males (76.7%) and adolescents (15.77 ± 0.84 years). It was possible to observe that 25 patients (58.1%) had a positive family history, while 18 patients (41.9%) had no history of the disease. Regarding eating habits, 7 patients (16.3%) consumed milk and 3 patients (7.0%) consumed chocolate, while 31 patients (72.1%) consumed fatty foods. Two individuals (4.7%) consumed foods high in sugar. Fourteen (32.6%) individuals fell into the mild and moderate acne categories, respectively, while 15 patients (34.9%) were included in the severe acne category. The median value for the level of acid free fatty acids was 24,358 ppm, with the PCR being positive for *P. acnes* in 5 patients (11.6%), and negative for 38 patients (88.4%).

The concentration of FFA in severe acne (median = 30,400 ppm) was significantly higher than in the mild degree of the condition (median = 12,746 ppm) ($p < 0.05$). The verified level of fatty acids in moderate acne was also significantly higher than in the mild degree. However, there was no significant difference in FFA levels among patients with moderate and severe

Table 1: Basic Characteristics of the population

Category	Frequency (n)	Percentage (%)
Gender		
Male	33	76.7
Female	10	23.3
Age (mean, SD)	15.77 ± 0.84 years	
Family history		
Present	25	58.1
Absent	18	41.9
Eating habits		
Milk	7	16.3
Chocolate	3	7.0
Fatty foods	31	72.1
Food rich in sugar	2	4.7
Acne severity degree		
Mild	14	32.6
Moderate	14	32.6
Severe	15	34.9
FFA levels (ppm) (median, IQR)	24.358 (12.946-39.838)	
***P. acnes'* PCR**		
Positive (+)	5	11.6
Negative (-)	38	88.4

acne (Figure 1). There was no significant difference between the level of FFA in patients with positive PCR who were negative for *P. acnes* (Figure 2).

DISCUSSION

The present study shows that the level of FFA influences the severity of acne vulgaris. This result is in line with another study that found evidence of an important role of *P. acnes* in the pathogenesis of acne through the release of lipase enzymes, that break down triglycerides into glycerol and free fatty acids, which cause inflammation and tissue damage.[5] Moreover, a review article also showed that fatty acid peroxidation products are capable of inducing inflammation through the activation of peroxisome proliferator-activated receptors (PPARs), with PPARα and PPARγ acting as the main isoforms.[12]

Data obtained in the present study showed that the higher the levels of palmitic acid in the face, the higher the degree of severity of acne. The levels of palmitic acid in the facial skin of patients with severe and moderate acne were found to be significantly higher than in those with mild degree. Katsuta et al. (2005) have shown that the application of FFA in the ears of rabbits and mice induces hyperkeratinization and epidermal hyperplasia similar to that of comedos formation.[5] In addition, it is suspected that FFA also affect follicular keratinocytes and epidermal lipid bilayer structures that may affect the occurrence of acne. This was demonstrated by Youn in a 2015 study in which a greater amount of sebum was found in the facial skin of patients with acne, nevertheless there was no analysis of the content of sebum.[13]

In contrast, an interesting result was shown in a recent clinical trial in which the administration of lymecycline led to an increase in FFA levels.[14] However, the pre- and post-treatment clinical condition was not studied, meaning that it was not possible to conclude that the presence of correlation with an improvement or worsening of the disease. One possible explanation for this difference is a deficiency in linoleic acid and α-linoleic acid in the patients evaluated in the present study, causing a compensation in the form of increased levels of palmitic and oleic acids.[15] Studies have suggested that linoleic acid deficiency may increase PGE- 2, a potent inflammatory mediator commonly found in patients with acne vulgaris.[16] This might explain the higher level of palmitic acid in the individuals with more severe acne who took part in the present study.

A different result was also described by Pappas (2009), when levels of FFA in patients with acne were low. In this study, the number of patients was limited and the authors did not explain how many individuals and controls participated in the analysis.[7] This difference might be caused by eating habits, for a low glycemic index diet has been found to be associated with increased levels of FFA and incidence of acne.[17]

Data from the present study showed that levels of FFA in patients with PCR-positive results for *P. acnes* did not differ significantly from those in patients with PCR-negative results. Similar conclusions were obtained by Akaza et al., where al-

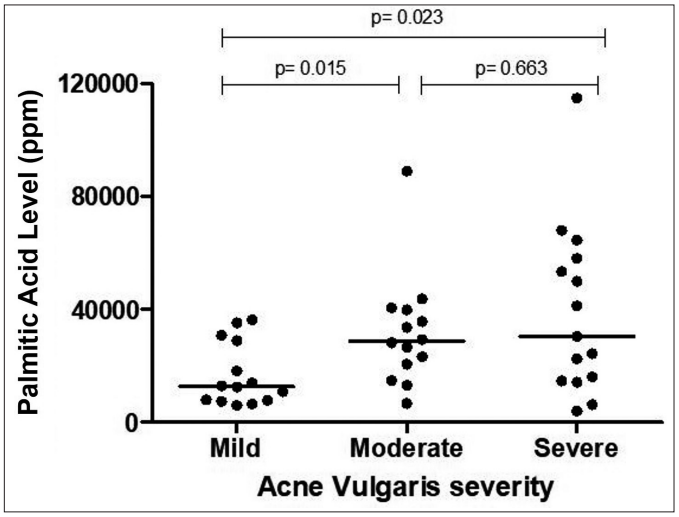

Figure 1: Comparison of FFA levels between different groups of acne vulgaris

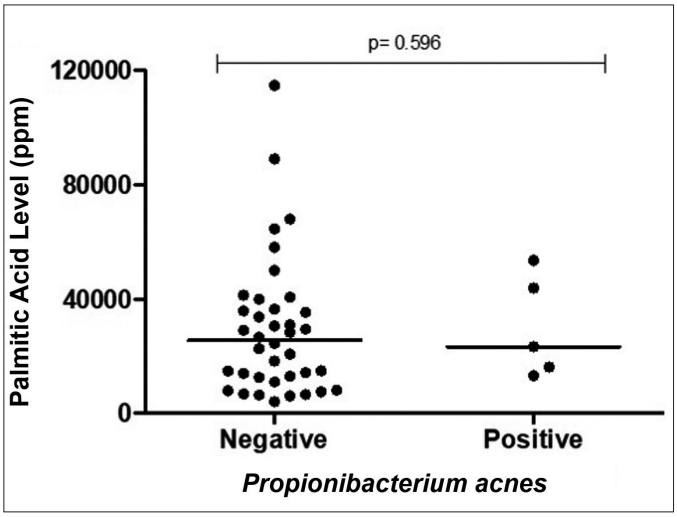

Figure 2: Association of FFA levels and P. acnes, evidenced by PCR

though FFA levels played a role in abnormal keratinization and induced comedo formation, the number of *P. acnes* in patients with and without acne were not found to be significantly different.[18] This may be due to other commensal bacteria, such as *S. epidermidis*, which can produce lipase, that in turn causes the hydrolysis of sebum triglyceride and produces FFA.[6] These findings suggested that there are other factors besides the levels of FFA that might affect the growth of *P. acnes*.

The present study corresponds to an initial analysis performed aimed at evaluating the prevalence of acne vulgaris in the adolescent population of an urban area and its association with the level of FFA, thus providing a foundation for future studies in this field. Also, the authors included only a sufficient number of participants to obtain a valid result. However, because it is a preliminary analysis, the present study was performed in only one population, implying that the study population was homogeneous.

CONCLUSION

The outcomes of the present study shows that FFA levels affect the degree of acne vulgaris and that the increase in the level of FFA was associated with the intensification of the severity of acne. Thus, FFA levels can be used as one of the markers for determining the severity of acne vulgaris. ●

REFERENCES

1. Degitz K, Placzek M, Borelli C, Plewig G. Pathophysiology of acne. J Dtsch Dermatol Ges. 2007;5(4):316-23.
2. Mourelatos K, Eady EA, Cunliffe WJ, Clark SM, Cove JH. Temporal changes in sebum excretion and propionibacterial colonization in preadolescent children with and without acne. Br J Dermatol. 2007;156(1):22-31.
3. Bhate K, Williams HC. Epidemiology of acne vulgaris. Br J Dermatol. 2013;168(3):474-85.
4. Zaenglein AL, Pathy AL, Schlosser BJ, Alikhan A, Baldwin HE, Berson DS, et al. Guidelines of care for the management of acne vulgaris. J Am Acad Dermatol. 2016;74(5):945-73.e33.
5. Katsuta Y, Iida T, Inomata S, Denda M. Unsaturated fatty acids induce calcium influx into keratinocytes and cause abnormal differentiation of epidermis. J Invest Dermatol. 2005;124(5):1008-13.
6. Nakatsuji T, Kao MC, Zhang L, Zouboulis CC, Gallo RL, Huang C-M. Sebum Free Fatty Acids Enhance the Innate Immune Defense of Human Sebocytes by Upregulating β-Defensin-2 Expression. J Invest Dermatol. 2010;130(4):985-94.
7. Pappas A. Epidermal surface lipids. Dermatoendocrinol. 2009;1(2):72-6.
8. Berg JM, JL T, L S. Biochemistry New York: W H Freeman; 2002 [citado em 1 Jul 2018]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22497/.
9. Zhou BR, Zhang JA, Zhang Q, Permatasari F, Xu Y, Wu D, et al. Palmitic Acid Induces Production of Proinflammatory Cytokines Interleukin-6, Interleukin-1, and Tumor Necrosis Factor- via a NF-B-Dependent Mechanism in HaCaT Keratinocytes. Mediators Inflamm. 2013;2013:530429.
10. Desbois AP. Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. Recent Pat Antiinfect Drug Discov. 2012;7(2):111-22.
11. Lehmann HP, Robinson KA, Andrews JS, Holloway V, Goodman SN. Acne therapy: a methodologic review. J Am Acad Dermatol. 2002;47(2):231-40.
12. Ottaviani M, Camera E, Picardo M. Lipid mediators in acne. Mediators Inflamm. 2010;2010.
13. Youn SW, Park ES, Lee DH, Huh CH, Park KC. Does facial sebum excretion really affect the development of acne? Br J Dermatol. 2005;153(5):919-24.
14. Costa A, Siqueira AT, Parra DCdO, Silva CP, de Souza ETW, Sabino de Matos L, et al. Evaluation of the Quantitative and Qualitative Alterations in the Fatty Acid Contents of the Sebum of Patients with Inflammatory Acne during Treatment with Systemic Lymecycline and/or Oral Fatty Acid Supplementation. Dermatol Res Pract. 2013;2013:120475.
15. Sardesai VM. The Essential Fatty Acids. Nutr Clin Pract. 1992;7(4):179-86.
16. Ziboh VA, Miller CC, Cho Y. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. Am J Clin Nutr. 2000;71(1 Suppl):361s-6s.
17. Smith RN, Mann NJ, Braue A, Makelainen H, Varigos GA. A low-glycemic-load diet improves symptoms in acne vulgaris patients: a randomized controlled trial. Am J Clin Nutr. 2007;86(1):107-15.
18. Akaza N, Akamatsu H, Numata S, Matsusue M, Mashima Y, Miyawaki M, et al. Fatty acid compositions of triglycerides and free fatty acids in sebum depend on amount of triglycerides, and do not differ in presence or absence of acne vulgaris. J Dermatol. 2014;41(12):1069-76

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