

Levels of IL-12, IL-17, and LL-37 in Acne Vulgaris

Akne Vulgaris'te IL-12, IL-17 ve LL-37 Düzeyleri

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Abstract

Introduction: Acne vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous unit with a multifactorial pathogenesis, which includes colonization of *Propionibacterium acnes* (*P. acnes*). Increased *P. acnes* colonization causes a Toll-like receptor (TLR)-2-dependent increase in the production of interleukin (IL)-12 and cathelicidin (LL-37), and a Th-17-dependent increase in interleukin (IL)-17. This study aimed to investigate the relationship between IL-12, IL-17, and LL-37 from patient's serum and various severities of AV.

Materials and Methods: This study was an analytic observational cross-sectional study. Subjects were enrolled using the consecutive sampling method and assigned according to the *Global Acne Grading System* (GAGS) criteria. Statistical analysis was performed with one-way analysis of variance and Kruskal-Wallis tests.

Results: Mean levels of IL-12, IL-17, and LL-37 in the serum in mild AV were 50.65±6.38, 119.07±24.61, and 180.26±112.92 IU/mL, respectively. The mean levels of IL-12, IL-17, and LL-37 in moderate AV were 47.82±6.51, 132.52±19.41, and 165.91±82.08 IU/mL, respectively. The mean levels of IL-12, IL-17, and LL-37 in severe AV were 48.78±4.93, 208.34±35.38, and 259.50±130.88 IU/mL, respectively. In very severe AV IL-12, IL-17, and LL-37 levels were 39.63, 251.29, and 113 IU/mL, respectively. There were no significant differences between the serum levels of IL-12 (p=0.157) and LL-37 (p=0.434) in the different severities of AV, whereas there was a significant association between the serum levels of IL-17 and the severity of AV (p<0.001).

Conclusion: IL-17 is associated with severity of acne vulgaris, while no-association was found between the severity of the disease and IL-12 or LL-37.

Keywords: Acne vulgaris, GAGS, IL-12, IL-17, LL-37

Öz

Giriş: Akne vulgaris, *Propionibacterium acnes*'i (*P. acnes*) de içeren bir grup bakterinin pilosebase ünitesi enfekte etmesi ile oluşan kronik yangı ile oluşmaktadır. Artan *P. acnes* kolonizasyonu toll benzeri reseptör (toll-like receptör; TLR)-2'ye bağlı interlökin (IL)-12, katelisinidin (LL-37) ve Th-17-ye bağlı IL-17 üretimini artırır. Bu çalışmada, değişik şiddette AV'li hastaların serumundaki IL-12, IL-17 ve LL-37 düzeylerinin ölçülmesi amaçlanmıştır.

Gereçler ve Yöntemler: Çalışma çapraz-kesit irdeleyici bir gözlem çalışmasıdır. Olgular, Global Akne Derecelendirme Sistemi ölçütlerine göre örneklenmiş ardışık hastalardır. İstatistiksel test olarak tek yönlü varyans ile irdelenmiş Kruskal Wallis testi kullanılmıştır.

Bulgular: Hafif şiddetteki AV olan hastalardaki serum IL-12, IL-17 ve LL-37 düzeyleri sırası ile 50.65±6.38, 119.07±24.61, and 180.26±112.92 IU/mL olarak saptandı. Orta şiddette AV'li olan hastalarda ortalama IL-12, IL-17 ve LL-37 seviyeleri sırası ile 47.82±6.51, 132.52±19.41 ve 165.91±82.08 IU/mL iken, bu değerler, ağır AV'li olan olgularda 48.78±4.93, 208.34±35.38, 259.50±130.88 IU/mL ve çok ağır AV'li hastalarda ise 39.63, 251.29 ve 113 IU/mL idi. Serum IL-12 (p=157) ve LL-37 (p=0.434) seviyeleri açısından gruplar arasında istatistiksel açıdan anlamlı bir fark yok iken, IL-17 seviyeleri bakımından aradaki fark istatistiksel açıdan anlamlı (p<0.001) bulundu.

Sonuç: Farklı şiddette AV olan hastalarda, serum IL-12 ve LL-37 hastalık şiddeti ile ilişkili değil iken IL-17 düzeyleri hastalık şiddeti ile ilişkili olarak bulunmuştur.

Anahtar Kelimeler: Akne vulgaris, Global akne derecelendirme ölçütü, IL-12, IL-17, LL-37

Introduction

Acne vulgaris (AV) is an inflammatory condition of the pilosebaceous unit. AV predilection is in skin areas with the densest population of sebaceous follicles, in face (99% of cases), upper chest (15%), and back (60%).^[1,2] AV is a commonly found in teenagers, affecting nearly 85%, although prevalence decreases with age. Although not clearly understood,

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Received: Dec 09, 2017

Accepted: Jun 10, 2018

<https://doi.org/10.25002/tji.2018.761>

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many researchers agree that AV pathogenesis is caused by four related factors, including increased sebum production, keratinocyte hyperproliferation in the pilosebaceous duct, inflammation, and activity of *Propionibacterium acnes* (*P. acnes*).^[3,4] The clinical appearance of AV varies, consisting of non-inflamed and inflamed lesions. There are various methods to assess the degree of severity of AV, including the *Global Acne Grading System* (GAGS), which divides AV into mild, moderate, severe, and very severe clinical forms.^[5] The *P. acnes* bacteria can activate the immune response by stimulating keratinocytes to initiate cytokine-driven pro-inflammation.^[4]

P. acnes colonization can also activate Toll-like receptor-2 (TLR-2) in monocytes, leading to interleukin (IL)-12 and IL-8 production.^[6] Observation of the infiltration of immune cells, especially CD4+ cells, in perifollicular lesions of early AV showed that T-helper cells may be involved in the immune response caused by intra-follicular colonization of *P. acnes*. A recent report showed that *P. acnes* could induce secretion of IL-17 by T cells, indicating that AV could be a disease mediated by Th-17 cells.^[7] A recent study indicated that *P. acnes* caused to release various proteases through the activation of protease-activated receptor-2 (PAR-2) in keratinocytes, which could then increase transcription of pro-inflammatory cytokines, including IL-1 α , IL-8, and tumor necrosis factor (TNF)- α , as well as matrix metalloproteinase (MMP) and cathelicidin (LL-37).^[6]

To date, the knowledge regarding the cytokine profile of AV lesion is very limited. Although recent studies have provided baseline information on the issue, their role on severity of AV at various degrees is yet to be explored. Here we studied serum levels of IL-12, IL-17, and LL-37 in AV patients in association with the severity of the disease.

Materials and Methods

There were 68 subjects in total. Thirtyeight patients had mild disease whereas 24 and 5 cases had moderate and severe AV. One patient had very severe disease. There were 32 male 36 female patients between 11 and 30 years of age. Body mass index of the patients varied between 15.76 and 25.71. There was no statistical significance in terms of age, BMI, gender and menstruation influence between mild, moderate, severe and very severe groups. Table 1 describes the baseline characteristics of the patients according to GAGS. This was an analytic observational cross-sectional study. The serum samples were collected from patients' peripheral blood. The degree of severity was determined using the GAGS The grading of the lesions was done by two physicians.

Inclusion criteria were the following; being registered in the Medical Cosmetic Division of Dermatovenerology Outpatient Clinic, General Hospital Saiful Anwar Malang; willing to participate to the study; and signed an informed consent. Exclusion criteria included having topical treatment for AV for past 2 weeks; pregnancy, lactation, menstruating, using hormonal contraceptive, having any systemic treatment or skin disorder (malignancy, autoimmune, inflammation); having body mass index (BMI) >24.9 kg/m². Level of IL-12, IL-17 and LL-37 in the patient's blood serum were measured by the ELISA method, with units of picogram/milliliter (pg/mL)

For statistical comparison, *one-way ANOVA* test was used for data showing normal distribution, and *Mann-Whitney* test was performed, using SPSS version 16.^[8]

Table 1. Baseline characteristics comparison of samples based on the degree of severity of AV

Baseline characteristics	Acne vulgaris degrees (GAGS)				p
	Mild	Moderate	Severe	Very Severe	
Age (years) (mean \pm Std. dev.)	22.4 \pm 4.5	20.13 \pm 4.4	19.4 \pm 2.7	26.0	0.116
BMI (mean \pm Std. dev.)	20.3 \pm 2.2	20.1 \pm 2.43	20.4 \pm 3.3	25.46	0.187
Gender (n= 68, frequency)					0.154
Male	19	8	4	1	
Female	19	16	1	0	
Affected by menstruation (only for female patients)					0.797
No (n= 11)	6	5	0	0	
Yes (n= 25)	13	11	1	0	

Table 2. Normality test of IL-12, IL-17, and LL-37 (one-sample Kolmogorov-Smirnov test)

		IL-12 Levels	IL-17 Levels	LL-37 Levels
N		68	68	68
	Mean ^{a,b}	49.3493	132.3	180.0
	Std. Deviation	6.5	35.8	104.8
Most Extreme Differences	Absolute	0.087	0.154	0.276
	Positive	0.087	0.154	0.276
	Negative	-0.060	-0.139	-0.155
Kolmogorov-Smirnov Z		0.719	1.271	2.279
Asymp. Sig. (2-tailed)		0.680	0.079	<0.0001

^aTest distribution is normal.
^bCalculated from data.

Results

Table 1 shows baseline characteristic comparison of samples according to GAGS.

Before comparison of IL-12, IL-17, and LL-37 levels based on AV degree of severity, normality and homogeneity tests of the data distribution was conducted. This assumption test would decide whether a parametric or non-parametric test was used.^[8]

The normality test suggested that IL-12 and IL-17 exhibited a normal distribution (Table 2). Since LL37 values were not distributed normally ($p < 0.001$), Kruskal Wallis test was used to analyze LL37 levels.

Levene's test was used for homogeneity of variances (Table 3). Comparison of IL-12, IL-17, and LL-37 according to the various degrees of severity of AV is depicted in Table 4. The only statistical significance between each degree of severity was found only for IL-17 level ($p < 0.001$)

Discussion

P. acnes has been expected initiating the inflammation process of acne. However, there are no supporting theories about its role in the inflammation pathway. This bacterium produces bioactive substances and enzymes as the chemo-attractant for non-specific immune system in various pathways. Furthermore, there are several pathways involved in the acne inflammation process such as antimicrobial peptides inducing pro-inflammation cytokines production.

The role of IL-12 in AV pathogenesis is related to the colonization of *P. acnes*.^[9] *P. acnes* bacteria proliferate at the pilosebaceous unit, inducing chemotactic factor release, including IL-12, leading to development of an inflamed lesion.^[10,11] There are several theories explaining this phenomenon, one of which is that the interaction of *P. acnes* with TLR-2 triggering innate and adaptive immune response signaling.^[12] When TLR-2 is activated, nuclear translocation of the transcription factor (NF- κ B), promotes expression of genes involved in the immune

Table 3. Homogeneity test of various levels of IL-12, IL-17, and LL-37

Variable	Levene test	Significance value	Result
IL-12 levels	0.209	0.812	Homogeneous
IL-17 levels	1.799	0.174	Homogeneous
LL-37 levels	1.481	0.235	Heterogeneous

Table 4. Comparison of mean levels of IL-12, IL-17, and LL-37 in various degree of severity of AV

Variable	AV Degree of Severity (mean \pm SD)				p
	Mild	Moderate	Severe	Very Severe (n=1)	
IL-12 levels	50.7 \pm 6.4	47.8 \pm 6.5	48.78 \pm 4.9	39.63	0.157
IL-17 levels	119.1 \pm 24.6	132.5 \pm 19.4	208.34 \pm 35.4	251.29	<0.001
LL-37 levels	180.3 \pm 112.9	165.9 \pm 82.1	259.50 \pm 130.9	113.00	0.434

response, such as gene modulating chemokines, cytokines, and adhesion molecules.^[13] Studies regarding bacteria induced tissue inflammation in experimental animals showed that macrophages released a two-fold greater amount of IL-12 p40 with response *P. acnes* compared with that of *Staphylococcus epidermidis*.^[9]

Patients who had various systemic and local treatments, which may have affected serum IL-12 levels, were excluded from the study. But since this study was based on history taking, there could be a selection bias therefore no significant differences in the different degrees of AV severity was found possibly due to the considerable number of confounding factors unknown in this study.

In the present study, there was a difference in mean serum IL-17 levels between the patient groups with different severity of AV. Mean serum IL-17 levels further increased in line with increasing AV severity. IL-17 plays a role in the development of inflamed lesions, where *P. acnes* is believed to be able to induce production of IL-17 and IL-22 in the peripheral area, as well as the IL-17 receptors, IL-17RA and IL-17RC.^[14] An immunogenic protein of *P. acnes* released in the follicle would be processed by Langerhans cells and presented to CD4⁺ cells. Cytokine secretion would cause differentiation of naïve CD4⁺T cells to Th-17 cells, which then would produce IL-17, resulting in the inflammation that is one of the characteristics of AV.^[7] Agak et al., stated that *P. acnes* isolated from AV patients were able to significantly ($p < 0.001$) induce IL-17 secretion in human peripheral blood mononuclear cells (PBMCs), with an average value of 500-700 pg/mL.^[7]

Furthermore, IL-17 plays a role in the proliferation and differentiation of keratinocytes. Lai et al., showed that after IL-17 bound the IL-17RA receptor in keratinocytes, the keratinocytes were sensitized to express regenerating islet-derived protein 3-alpha (REG3A).^[15] The REG3A protein provides feedback to keratinocytes to inhibit termination of differentiation processes, and increases cell proliferation through binding with exostosin-like 3 (EXTL3), followed by activation of phosphatidylinositol 3-kinase (PI3K). While in other studies, the roles of IL-17 were determined via stress-activated protein kinase (SAPK) signaling, therefore causing hyperproliferation and disturbing keratinocyte differentiation.^[15]

The mean serum LL-37 levels were not changed according to severity of AV ($p = 0.43$). The role of LL-37 in AV has

yet to be elucidated. A study conducted by Lee et al., demonstrated that LL-37 could kill skin microfloral bacteria, such as *Staphylococcus aureus* or *P. acnes*, *in vitro*, but this was only relevant when LL-37 increased with simultaneous administration of with other antimicrobial agents.^[15] Based on that study, it was shown that there were only two antimicrobial combinations, that is LL-37 and psoriasis, which resulted in a synergistic effect against *P. acnes*.^[16] A study by Rahadini described a statistically significant correlation between LL-37 levels and the degree of severity of AV ($r = 0.256$, $p = 0.027$).^[17] Therefore, it could be concluded that there is a significant relationship between LL-37 serum levels and the degree of severity of AV.

Cathelicidin or LL is one of the antimicrobial peptides produced in human with size of 4.5 kDa as a results of hCAP-18 activation by infection, trauma, and inflammation.^[18] LL-37 is found in tissues and in the circulation. Circulating LL-37 is especially affected by the expression of LL-37 by neutrophils, which are the main producer of LL-37.^[19] The mechanism of LL-37 induction in tissues or in circulation has not yet been determined. LL-37 levels showed low expression in the healthy skin but could be induced during skin inflammation and local or systemic infection, such as psoriasis vulgaris, rosacea, lupus erythematosus, hidradenitis suppurative, or rheumatoid arthritis.^[20] Several other inflammatory conditions, especially on epithelial surfaces and the mucosa, could affect LL-37 expression, such as periodontitis, as well as gastrointestinal, urogenital, and respiratory tract infections.^[21,22] LL-37 expression was maintained in a balanced condition, to preserve homeostasis.

Furthermore, the upregulation of LL-37 in AV lesion using *P. acnes* activated the alarmin leading to increase of host immune cells and pro-inflammation mediator secretion. As the result, the severity level of AV increases. However, the activity of LL-37 has also been revealed as anti-inflammatory as a modulator of its pro-inflammatory activity.^[22]

In this study, there was no significant association between serum IL-12 and LL-37 levels and the degree of severity of AV, but there was a significant association between serum IL-17 levels and the degree of severity of AV, according to GAGS classification. It can be suggested that a study about the relation of IL-12, IL-17, and LL-37 relation in AV severity level might be performed to investigate the other cofounding factors.

Acknowledgements: Authors thank to Brawijaya University and Saiful Anwar General Hospital Malang for facilitated this research.

Ethics Committee Approval: This study has been approved by Ethical Committee Medicine Faculty, Brawijaya University Malang No. 400/3/K.3/302/2016 -> for parameter IL-7, 400/111/K.3/302/2015 -> for parameter LL-37, 400/114/K.3/302/2015 -> for parameter IL-12

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept - SM; Design - SM; Supervision - SM; Resource - YK, YM, ER; Materials - YK, YM, ER; Data Collection and/ or Processing - YK, YM, ER; Analysis and/or Interpretation - SM; Literature Search - YK, YM, ER; Writing - SM, YK, YM, ER; Critical Reviews - SM

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