The Difference in Interleukin-12 (IL-12) on Degrees of Acne Vulgaris Severity

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Abstract

Introduction: Acne vulgaris (AV) is a common disorder of pilosebaceous seen mainly in adolescents. Most cases of AV present with a pleomorphic lesion array, consisting of blackheads, papules, pustules, and nodules of varying severity. Acne is one of the three most common skin disease, especially in adolescents and young adults, with an estimated prevalence of 85% (aged 12-25 years). Acne vulgaris (AV) is still a health problem in the world and Indonesia. The causes of AV are multifactorial and can lead to various complications. **Aim**: This study looked for the difference in interleukin-12 (IL-12) on degrees of AV severity. **Materials and Method**: This study was an observational analytic study with a cross-sectional approach. The research was conducted at the Diniyah Putri Islamic Boarding School, Lampung Province, Indonesia, in August 2020. The independent variable in this study was the AV degree, while the dependent variable was the IL-8 level. The sampling method in this study was consecutive sampling, as many as 63 AV patients. The research material is serum from venous blood. Examination of interleukin-8 levels using the ELISA method. Data analysis was univariate to find the mean and standard deviation of IL-8 levels, as well as the frequency distribution to determine the degree of AV. While the bivariate analysis was to determine the difference in the mean of IL-8 levels in AV patients. **Results**: The mean age of the respondents was 15.49 ± 1.07 years, the mean IL-12 level was 48.22 ± 15.62 and most of the AV degrees were in a severe category as much as 61.9%. **Conclusion**: Statistically there is no difference in the mean IL-12 levels in moderate and severe AV patients, but IL-12 levels are higher at severe AV degrees than moderate AV degrees.

Keywords*: a degree of acne vulgaris, IL-12 levels*

Introduction

Acne vulgaris (AV) is a common disorder of pilosebaceous seen mainly in adolescents. Most cases of AV present with a pleomorphic lesion array, consisting of blackheads, papules, pustules, and nodules of varying severity.[1] Blackhead are the primary acne lesion. Patients usually will have lesions of various forms.[2] AV is difficult to cure and requires a longer treatment time.[3] AV is still a problem in the world and Indonesia.[4,5] Acne is one of the three most common skin disease, especially in adolescents and young adults, with an estimated prevalence of 85% (aged 12-25 years).[6] AV attacks the population of adolescents and young adults in the world.[7] AV in Lampung Province is more experienced by women (69.7%) than men (30.3%). Young people (16-25 years) experienced acne vulgaris 53.2% more. Cosmetics users were more likely to experience acne vulgaris (59.1%).[8] AV is a multifactorial condition, which is caused by various factors[9,10] AV lesions can be divided into non-inflammatory lesions in the form of blackheads, and inflammatory lesions in the form of papules, pustules, and nodules.[11] This disease also has psychosocial effects.[12] AV has unknown pathogenesis, but it is thought to be multifactorial and interrelated.[13] Inflammation is an important factor in the pathogenesis of AV, especially induced by immunological reactions to Propionibacterium acnes (P. acnes).[14]

P. acnes belongs to the Corynebacteria group of bacteria. P. acnes is an anaerobic diphtheroid that usually persists in normal skin. These bacteria participate in the pathogenesis of acne by producing lipases, which break free fatty acids from skin lipids. These fatty acids can

cause tissue inflammation and contribute to acne.[15]

P. acnes is part of the normal skin flora, occasionally these bacteria appear in blood cultures and must be distinguished as an actual cause of disease. An important feature of P. acnes is the irregular rod shape seen on gram-positive stains. These bacteria can grow in the air and do not produce endospores. These bacteria can be in the form of branched filaments or a mixture of rods or filaments with a coccoid shape. P. acnes requires oxygen ranging from aerobic or facultative anaerobes to microfilic or anaerobes. Some are pathogenic for animals and plants. Medicines that are used topically contain mostly trace elements of sulfur and other astringents.[16]

AV generally occurs at puberty. In women, AV can be the first sign of puberty and can occur one year before menarche. The peak prevalence often occurs in childhood to late adolescence and affects adults. The incidence of AV then decreases with age, but it can persist.[17]

Four main factors play a role in the pathogenesis of AV, namely follicular epidermal hyperproliferation, excessive sebum production, inflammation, presence and activity of P. acnes.[18] The first factor is follicular epidermal hyperproliferation to produce blackheads. The upper epithelium of the hair follicle (infundibulum), becomes hyperkeratotic with increased cohesion of keratinocytes, causing blockage of the follicular estuary. The stimulation of keratinocyte hyperproliferation and increased adhesion is not known, but several factors are suspected, namely stimulation of androgen hormones, decreased linoleic acid, increased IL-1α activity, and the effect of P. acnes.

The pathogenesis of AV is the excessive production of sebum. Sebum components in the form of triglycerides and lipoperoxidase play an important role in the pathogenesis of acne. Triglycerides are broken down into free fatty acids (FFA) by P. acnes, which is the normal flora of sebaceous follicles. These fatty acids then encourage the colonization of P. acnes.[19]

Lipoperoxidase produces proinflammatory cytokines and activates the peroxisome proliferator-activated receptor (PPAR) pathway, which increases sebum. Micro-comedones will continue to grow with dense keratin, sebum, and bacteria which eventually causes the follicle wall to break. This process rapidly stimulates the inflammatory process. Within 24 hours of follicular wall rupture, lymphocytes immediately collect, CD4+ lymphocytes are found around the hair follicle, while CD8+ around the perivascular area. One to two days after blackhead rupture, neutrophils become the dominant cells around the micro comedo.

The cell wall of P. acnes contains carbohydrate antigens which stimulate the formation of antibodies. Anti-propionibacterium antibodies enhance the inflammatory response by activating the proinflammatory cascade. Propionibacterium acnes plays a role in the pathogenesis of acne by producing lipase, protease, and hyaluronidase enzymes which are important for converting triglycerides into free fatty acids which play a role in the inflammatory process and secrete chemotactic factors.[20]

AV treatment is based on its pathophysiology, namely improving follicular keratinization, reducing sebaceous gland activity, reducing the number of bacterial populations, especially P. acnes, and reducing inflammation. AV treatment is given based on the degree of severity, AV degrees of mild, moderate, and severe. This treatment can be in the form of topical therapy and systemic therapy.[21]

The appearance of AV lesions is due to the presence of pro-inflammatory lipids and cytokines. P. acnes is thought to mediate the advanced inflammatory process. The role of cytokines in the pathogenesis of AV can be identified because there is an inflammatory process from the beginning to the end of the development of AV lesions. The inflammatory process occurs mainly because it is induced by an immunological response from the host to P. acnes.[22]

P. acnes also stimulates Toll-Like-Receptor-2 (TLR-2) on monocytes and polymorphonuclear (PMN) cells around sebaceous follicles. The stimulation of TLR-2 by P. acnes will increase the concentration of proinflammatory cytokines, especially Interleukin-8 (IL-8), Interleukin-12 (IL-12), and Tumor Necrosis Factor-Alpha (TNF-α). Activation of Toll-Like Receptors (TLRs) that induces TLR-2 by P. acnes on monocytes

will cause the release of cytokines Interleukin-12 (IL-12) and inflammatory cells involved in the pathogenesis of AV.[23] This study aimed to find differences in the mean IL-12 levels in moderate and severe AV.

Materials and Method

This research is an observational analytic study with a cross-sectional approach. The study was conducted at the Diniyah Putri Islamic Boarding School, Lampung Province, Indonesia, in August 2020. The study population was all patients suffering from moderate and severe AV. The sampling method in this study was consecutive sampling, all subjects who came and met the selection criteria were included in the study until the required number of subjects was met.

The independent variable in this study is the degree of AV. While the dependent variable is the level of IL-12. The research material is serum from venous blood. Blood sample centrifugation is carried out in the polyclinic, placed on a shelf, and put in a refrigerator. The blood containing 10 ml in the EDTA tube was taken to the Prodia Lampung Laboratory to be tested for IL-12 levels by the ELISA method using the Human IL-12 Immunoassay Quantikine ELISA kit®. The reading of the results and interpretation is carried out by the Laboratory of Prodia Lampung. All processes for establishing quality assurance in this study will refer to the procedures issued by the Center for Health Laboratories, Ministry of Health of the Republic of Indonesia. Data processing is carried out by the process of editing, coding, processing, and cleaning the data processed by computerized. Data analysis was mean on age and IL-2 levels as well as the percentage of moderate and severe AV patients.

Results and Discussion

Table 1. Characteristics of Respondents

Variable	Mean	Standard Deviation
Age	15,49	1,07
IL-12 Level	48,22	15,62

The results showed that the mean age of the respondents was 15.49 years with a standard deviation of 1.07 and a mean IL-12 level of 48.22 with a standard deviation of 15.62. The results showed that all of the respondents were adolescents. All respondents in this study were young women. AV can affect all ages, but most affect adolescents and young adults and can persist into middle age.[24]

Table 2. Degrees of AV Severity

AV Degree	Frequency	Percentage
Moderate	24	38,1
Severe	39	61,9
Total	63	100,0

The results showed that there were 24 patients (38.1%) in the moderate category and 39 patients (61.9%) in the severe category. The pathogenesis of AV is multifactorial, but four main factors play an important role in the pathogenesis of AV, namely increased sebum production, follicular hyperkeratinization, P. acnes colonization, and inflammation.[25]

Table 3. The Difference in IL-12 on Degrees of AV Severity

Degrees of AV Severity	Mean	Standard Deviation	p-value
Moderate	43,59	13,67	0,064
Severe	51,07	16,22	

The mean IL-12 levels in moderate AV patients was 43.59 with a standard deviation of 13.67. While the mean IL-12 levels in severe AV patients was 51.07 with a standard deviation of 16.22. further analysis obtained p-value = 0.064, which means that there is no difference in the mean IL-12 levels between AV degrees. But substantially, the mean IL-12 levels in severe AV patients were higher than IL-12 levels in moderate AV patients.

AV is a follicular disease that occurs most often and affects the area containing the largest sebaceous gland follicles, including the face, back, and body. AV is a multifactorial condition. AV lesions can be divided into non-inflammatory lesions in the form of comedones, and inflammatory lesions in the form of papules, pustules, and nodules. The pathogenesis of AV is multifactorial, but four main factors play an important role in the pathogenesis of AV, namely increased sebum production, follicular hyperkeratinization, P. acnes colonization, and inflammation.[26]

The combined acne severity classification was classified as mild acne, if <20 comedones, or <15 inflammatory lesions, or the total number of lesions were <30; moderate acne, if 20-100 blackheads, or 15-50 inflammatory lesions, or the total number of lesions is 30-125; severe acne, if >5 cysts, or total number of blackheads >100, or total inflammatory lesions >50, or total number of lesions >125.[27]

Research on levels of IL-12, IL-17, and LL-37 in AV on 68 subjects, which were divided into 38 people with mild AV, 24 people with moderate AV, and 5 people with severe AV. One patient had a very severe disease. There were 32 men and 36 women. Age between 11 and 30 years. The patient's body mass index varied between 15.76 and 25.71. There was no significant relationship between age, BMI, sex, and menstruation between mild, moderate, severe, and very severe groups. The analysis found no significant relationship between serum IL-12 and LL-37 and AV severity, but there was a significant relationship between serum IL-17 levels and AV severity.[28] Research on acne and serum levels of pro-inflammatory cytokines, found that the group of mice exposed to acne had higher levels of IL-12 than the group of mice that were not exposed to acne.[29]

Research on serum IL-12 levels in patients with AV inflammation and its correlation with severity in 27 AV patients, found that IL-12 levels in the acne group were higher than in the control group. The study found no significant relationship between IL-12 levels and AV severity.[30]

Inflammation in AV is caused by an increase in IL-12, a type of cytokine normally secreted by DC, MAC, and B lymphoblastoid cells, in response to antigen stimulation. IL-12 is also known as a T cell stimulant factor because it plays a role in the differentiation of CD4 T cells into TH1 cells. Effector T cells that produce IL-12 are called CD30 T cells. IL-12 is also a stimulant for the cytokines IFN-γ and TNF-α. IFN-γ stimulation is carried out by reducing the effect of the cytokine IL-4 which is the IFN-regulator. Furthermore, the production of IFN-γ will increase the level of IP-10 which is anti-angiogenic (inhibits the growth of new blood vessels).[31]

P. acnes is an anaerobic bacteria, gram-positive, has a peptidoglycan component that can trigger a cytokine response through TLR-2, which can cause tissue damage. P. acnes directly stimulate peripheral blood mononuclear cells (PMN) and monocytes to produce cytokines such as TNF-α, IL-1β, IL-12 via TLR-2. Activation of TLR-2 due to P. acnes on monocytes, leading to the release of cytokines IL-12 and other Toll-Like Receptors

(TLRs) and inflammatory cells may be involved in the pathogenesis of acne. The production of cytokines IL-12 is one of the main proinflammatory cytokines produced by monocytes in response to gram-positive organisms. Overproduction of IL-12 is present in several inflammatory diseases involving tissue damage.[32]

Interleukin-12 acts as a stimulating factor on Th1 cells. P. acnes induces TLR-2-induced IL-12 from peripheral blood monocytes and mononuclear. Because IL-12 is associated with AV inflammation. Research on serum IL-12 levels in patients with AV found that IL-12 levels in AV patients were significantly higher than in the control group.[33] Based on the results of the study, it was found that IL-12 levels in the AV patient group were higher than the control group. IL-12 levels in severe AV patients were higher than IL-12 levels in moderate AV patients.

Conclusion

Based on the results of the study, it was concluded that the mean age of the respondents was 15.49 years with a standard deviation of 1.07 and a mean IL-12 level of 48.22 with a standard deviation of 15.62. AV patients in the moderate category were 24 people (38.1%) and in the severe category were 39 people (61.9%). The mean IL-12 levels in moderate AV patients was 43.59 with a standard deviation of 13.67. While the mean IL-12 levels in severe AV patients was 51.07 with a standard deviation of 16.22. Further analysis obtained p-value = 0.064, which means that there is no difference in the average value of IL-12 levels between AV degrees. Substantially, the mean IL-12 levels in severe AV patients were higher than IL-12 levels in moderate AV patients.

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