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Interleukin-1 Alpha Expression In Men With Comedonal, Papulopustular, And Nodular Acne Vulgaris Lesions

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Abstract

Introduction : Acne vulgaris (AV) is a chronic, multifactorial pilosebaceous unit disorder, which generally appears in adolescence.[1] This condition causes non-inflammatory lesions, including: closed comedones or whiteheads and open comedones or blackheads and inflammatory lesions, such as papules, pustules, and nodules.[2] The main hypotheses regarding the pathophysiology of acne include: keratinization of the follicles in the sebum unit of hair follicles, colonization of Cutibacterium acnes (C. acnes) and follicle activation, hormone action, sebum production and release of inflammatory mediators.[6] Mechanisms of the inflammatory response against C. acnes will induce monocytes and other cells to produce interleukin (IL) namely IL-1α, IL-1β, IL-6, IL-8, IL-12, interferons (IFN), chemotactic factor, β-

defensin, tumor necrosis factor alpha (TNF-α), and other polypeptides and cytokines that can trigger an inflammatory response.[7]
Interleukin-1 alpha (IL-1α) and TNF- are involved in the initiation and maintenance of inflammation and immune responses in AV disorders.[8]

Purpose of the Study: This study aims at proving whether the expression of IL-1α in acne vulgaris lesions is directly proportional to the degree of acne vulgaris.

Methods: This study was an observational study with an analytical cross-sectional design to determine the expression of IL-1α in acne vulgaris lesions. Measurements were carried out once with a sample of 33 using purposive sampling technique. Punch biopsy was performed on the AV lesion area and then examined using immunohistochemistry with IL-1α primary antibody. The interpretation of the results of the immunohistochemistry examination was carried out by Anatomical Pathologists with 4 scales of brownish color intensity in the tissue, namely negative, weak, moderate, and strong. The data were analyzed using the Chi Square test.

Results and Discussion: The minimum IL-1α value for comedonal lesions was 3.04%, the maximum was 55.96%, the median value was 31.89% and the mean±SD was 32.81±17.70%. In papulopustular lesions, the minimum value was 18.77%, the maximum value was 63.11% with a median of 50.84% while the mean value was 47.16±14.77. For nodular lesions, the minimum value was 17.13%, the maximum value was 64.28%, with a median value of 34.61% and a mean value of 34.81±13.85%. The value of IL-1α expression by immunohistochemistry examination was weak (<25%) in 4 samples (30.8%) of comedonal AV lesions, 1 sample of moderate IL-1α expression (10.0%) and strong IL-1α expression were found in 3 samples (23.1 %). In AV papulopustular lesions, IL-1α was weakly displayed in 1 sample (10%), moderate IL-1α expression was displayed in 4 samples (40%) and IL-1α was strongly displayed in 5 samples (50.0%). As for nodular lesions, 2 samples (20.0%) showed weak IL-1α, 7 samples (70%) moderately displayed and 1 sample (10%). P value = 0.085> 0.05 which means there is no significant difference between IL-1α expression and AV lesions. **Conclusion**: There is no significant difference in the expression of IL-1α that appears in various degrees of acne vulgaris lesions.

Keywords: acne vulgaris, IL-1α, immunohistochemistry

Introduction

Acne vulgaris (AV) is a chronic, multifactorial pilosebaceous unit disorder that generally appears in adolescence.[1] This condition causes a disorder of non-inflammatory lesions, including closed comedones or whiteheads and open comedones or blackheads and inflammatory lesions, such as papules, pustules, and nodules.[2]

Global Burden of Skin Diseases in 2013 showed that AV accounted for 0.29% of all skin diseases and accounted for 1.79% of various forms of the world's disease.[3] Acne vulgaris affects about 80-100% of the population, in the age range of infants to the elderly, with the highest age peaks being women aged 14-17 years and men aged 16-19 years[4]. The prevalence rate of AV in adults is reported to be 64% at the age around 20 and 43% at age around 30. The incidence of AV tends to decrease and usually disappears by the end of the second decade or by the beginning of the third decade (20 - 30 years old).[5]

The main hypotheses regarding the pathophysiology of acne include: follicular keratinization in the sebum unit of hair follicles, colonization of Cutibacterium acnes (C. acnes) and follicle activation, hormone action, sebum production and release of inflammatory mediators.[6] The mechanism of the inflammatory response against C. acnes will induce monocytes and other cells to produce interleukin (IL) namely IL-1α, IL-1β, IL-6, IL-8, IL-12, interferon (IFN), chemotactic factor,β-defensin, tumor necrosis factor alpha (TNF-α), as well as other polypeptides and cytokines that can trigger an inflammatory response.[7]

Interleukin-1 alpha (IL-1α) and TNF-α are involved in the initiation and maintenance of inflammation and immune responses in AV disorders.[8] Duct keratinocytes stimulated by C. acnes will produce IL-1α cytokines that cause hyper cornification and abnormal growth in the pilosebaceous units (PSUs) from the infundibulum similar to come done formation. Local macrophages, keratinocytes and neutrophils will also produce IL-1α.[9]

The role of IL-1α in acne vulgaris

Interleukin-1 plays a central role in the regulation of inflammation and the immune response in acne vulgaris. Interleukin-1 alpha is produced under homeostatic conditions, but in comedones the levels produced reach 100 pg/mg which is a production level that supports the proinflammatory process[14]. There is evidence that IL-1α expression and secretion are dramatically increased during the early phase of acne formation[15].

Increased activity of the proinflammatory cytokine IL-1 is found at the early of hyperproliferation around uninvolved follicles and is thought to trigger activation of keratinocyte proliferation[16]. IL-1 production is initiated by injured keratinocytes and activates paracrine signals, attracts various lymphocytes, triggers selectins, and fibroblast migration. This production will also attract GM-CSF, TNFa, ICAM-1, and integrins. All of these cytokines will further activate and maintain hyperkeratinization[17].

Interleukin-1α is comedogenic which can cause hyper cornification of the infundibulum, as seen in comedone formation[15].

Comedones are caused by abnormal proliferation and differentiation of keratinocyte ducts. During the forming of acne there are two changes in the normal pattern of keratinization: 1) excessive proliferation of keratinocytes arranged in the follicle wall, as indicated by an increase in the cell proliferation marker Ki-67; 2) caused by decreased desquamation/exfoliation and increased cohesion between keratinocytes. These changes cause keratinocytes to accumulate in the follicle[15].

Assessment of IL-1α and TNF- expression was carried out by increasing the intensity of immunohistochemical staining using IL-1α and TNFα antibodies and histopathological readings by pathologists.[10]

Purpose of the Study

This study aims at demonstrating whether the expression of IL-1α in acne vulgaris lesions is directly proportional to the degree of acne vulgaris.

Research methods

The study was an observational study with an analytical cross-sectional design to determine the expression of IL-1α in acne vulgaris lesions. Measurements were carried out once. This study was conducted at the Cosmetic Dermatology Polyclinic, RSUD Dr. Moewardi Surakarta. Histopathological examination of skin tissue was carried out at the Anatomical Pathology Laboratory, Faculty of Medicine, Sebelas Maret University, Surakarta.

The total research subjects were 33 people consisting of all men. Subjects have met the inclusion and exclusion criteria and had an initial examination to determine the diagnosis of acne vulgaris. Inclusion criteria in this study included subjects with facial and non-facial acne vulgaris who had not received therapy and had received washout therapy for 4 weeks, aged at least 14-35 years old and willing to fill out informed consent.

Exclusion criteria in this study were subjects who had autoimmune or inflammatory diseases, pregnant women, breastfeeding mothers, obesity, and smokers.

Data Collection Procedure

1) The subjects with inclusion criteria were given explanation regarding the procedure, purpose, then asked to sign an informed consent.
2) The history taking, clinical examination, diagnosis of AV with mild and moderate grade lesions was performed based on clinical examination with GAGS score (mild grade: 1-18 and moderate: 19-30).
3) The study used a purposive sampling technique, researchers and research subjects did not know whether the subject was in the treatment or control group.
4) The patients who were given a biopsy of the plong with a diameter of 3 or 5 mm performed on the skin containing the specified acne lesions, previously given injection anesthesia, namely by injection of 2% lidocaine HCL.
5) Each acne lesion was examined for IL-1α expression and TNF- expression by making two slides of acne lesions using the polyelisen method for immunohistochemistry examination.

6) The examination results were recorded, documented, and analyzed.
7) After the biopsy, the skin area was given Gentamicin ointment, closed, and monitored for 2 weeks for re-control. The patient is given mefenamic acid which can be taken 3 times a day if in pain and is advised to return for treatment if the post-biopsy wound is worsen (increasing pain or not healing).
8) After 2 weeks the wound was reassessed for scarring. If a scar formed, further treatment was given.

Subjects underwent a long biopsy of the AV lesion area and then examined using immunohistochemistry with IL-1α primary antibody. The interpretation of the results of the immunohistochemistry examination was carried out by Anatomical Pathologists with 4 scales of brownish color intensity in the tissue, namely negative or not displayed, positive +1, which means that brown color is weak (<25% which is positively stained on the cytoplasmic membrane), positive +2, which is brown color moderately displayed (25-50% which is stained positively on the cytoplasmic membrane), and positive +3 that is brown color strongly appeared (>50% which is positively stained on the cytoplasmic membrane). The data were analyzed using the Chi Square test.

Results

This study was an observational study with an analytical cross-sectional design to determine the expression of IL-1α and TNFα in acne vulgaris lesions. The measurement was carried out once. This research was conducted at the Cosmetic Dermatology Polyclinic, RSUD DR. Moewardi Surakarta and histopathological examination of skin tissue was carried out at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret Surakarta from October 2021 to February 2022.

The calculation of the expression of IL-1α and TNF-α respectively using the immunohistochemistry technique with primary antibodies IL-1α and TNF-α (Wuhan Fine Biotech Co., Ltd, China). The interpretation of the results of the CPI examination was carried out by Anatomical Pathologists with 4 scales of brownish color intensity in the tissue, namely negative or not displayed, positive +1, which means that brown color is weak (<25% which is positively stained on the cytoplasmic membrane), positive +2, which is brown color is moderately displayed (25-50% which is stained positively on the cytoplasmic membrane), and positive +3 that is brown color strongly appeared (>50% which is positively stained on the cytoplasmic membrane).

The expression of IL-1α and TNF-α were respectively identified based on the appearance of immunoreactive cells with a magnification of 40 times using an Olympus CX 22 microscope RFS-1 model and a 5.0-megapixel Optilab CMOS camera. This study is aimed at determining whether there are differences in the expression of IL-1α and TNF- in various degrees of acne vulgaris lesions in comedonal, papulopustular and nodular lesions. Before testing the hypothesis, a description of the results of the research data was carried out, namely the expression of IL-1α and TNF-α at various degrees of acne vulgaris lesions in comedonal, papulopustular and nodular lesions. The number of research samples is 33 research subjects

Characteristics Description of Patient being Studied

The descriptive explanations of research patients are intended to obtain a more complete picture regarding the characteristics of the patients being studied.

Table 4.1. Characteristics of Research Subjects.

Characteristics	Total Number	Percentage (%)
Gender		
Male	33	100,1%
Age		
10-20 years old	3	9,1%
21-30 years old	27	81.8%
31-40 years old	3	9,1%

Job		
Cleaning Service	24	72.7%
Security	7	21.2%
Private Employee	2	6.1%
GAGS Score		
Light	18	54.5%
Moderate	15	45.5%
Lesions		
Comedonal	11	33.3%
Nodular	11	33.3%
Papulopustular	11	33.3%

Based on table 4.1 above, from a sample of 33 subjects, all genders were male (100%). Most of them are between 21-30 years old, namely 27 people (81.8%), between 10-20 years old and between 31-40 years old are 3 people (9.1%). The occupation of the research subjects was dominated by cleaning service, as many as 24 people (72.7%) then security guards as many as 7 people (21.2%) and private employees as many as 2 people (6.1%). Subjects who have GAGS scores in the light category are 18 people (54.5%) and 15 people are in the moderate category (45.5%). While the types of comedonal, nodular and papulopustular lesions were 11 people (33.3%).

The minimum IL-1α value for comedonal lesions was 3.04%, the maximum was 55.96%, the median value was 31.24% and the mean ± SD value was 31.24±18.44%. Papulopustular lesions obtained a minimum value of 17.13%, a maximum value of 64.28% with a median of 33.72% while the mean value of 34.55±13.16%. For nodular lesions, the minimum value was 18.77%, the maximum value was 63.11%, with a median value of 51.06% and a mean value of 47.52±14.07% (Table 4.1)

Table 4.1. The IL-1α value was based on 40 times magnification using an Olympus CX 22 microscope RFS-1 model and a 5.0-megapixel Optilab CMOS camera.

IL-1α (%)	Minimum	Maximum	Median	Mean ± SD
Comedonal Lesions	3,04	55,96	31,24	31,24±18,44
Nodular Lesions	18,77	63,11	51,06	47,52±14,07
Papulopustular Lesions	17,13	64,28	33,72	34,55±13,16

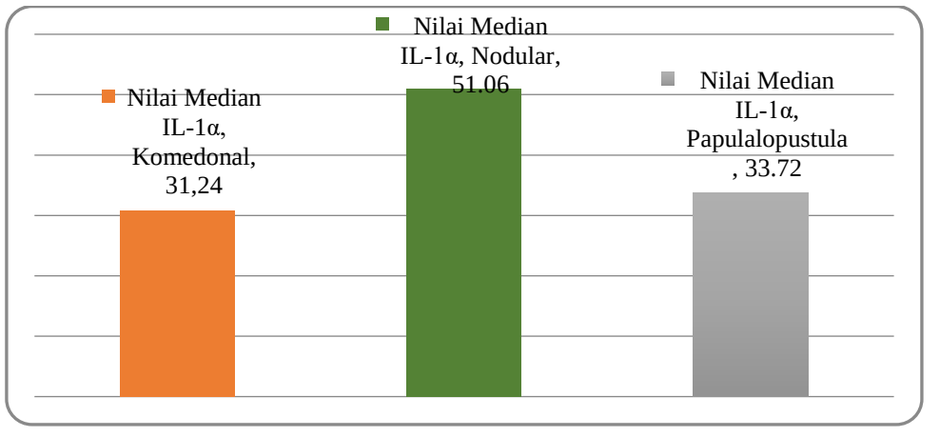

Figure 4.1. Graph of median value of IL-1α (%)

Based on the results of the chi square test in table 4.3. above, the value of IL-1α expression with immunohistochemistry examination showed weak (<25%) in comedonal AV lesions in 4 samples (36.4%), moderate IL-1α expression in 4 samples (45.5%) and strong IL-1α expression in 2 sample (18.2%). In AV nodular lesions, IL-1α was weakly displayed in 1 sample (9.1%), IL-1α expression was moderately displayed

in 4 samples (36.4%) and IL-1α was strongly displayed in 6 samples (54.5%). As for papulopustular lesions, 2 samples showed weak IL-1α (18.2%), 8 samples showed moderate (72.7%) and 1 sample showed strong (9.1%), with p value = 0.08> 0.05 which means there is no difference which is significant between IL-1α expression with AV lesions.

Table 4.3. The differences between IL-1α expression and AV lesions

Experssion IL-1α	AV Lesions			P
	Comedonal	Nodular	Papulopustular	
Weak	4 (36.4%)	1 (9.1%)	2 (18.2%)	0.085
Moedrate	5 (45.5%)	4 (36.4%)	8 (72.7%)	
Strong	2 (18.2%)	6 (54.5%)	1 (9.1%)	

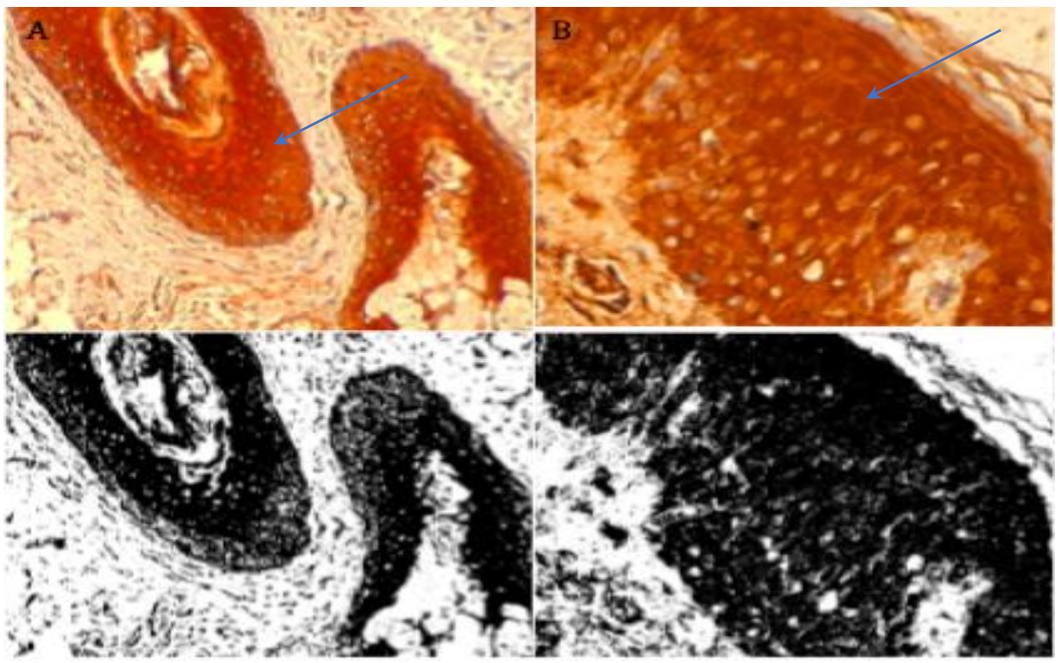

Figure 4.3. Expression of IL-1α at 10x (A) and 40x (B) magnification that appears at various degrees of acne vulgaris lesions is indicated by brownish color (blue arrow).

In Figure 4.3 with the Image J program. The results of the analysis are expressed in quantitative values (percentages) with the results of IL-1α expression. It appears that the strongest expression is in AV nodular lesions at 54.5%, comedonal at 18.2%, and papulopustular lesions at 9.1%.

Discussion

The results of this study showed that there was no significant difference in the expression of IL-1α that appeared at various degrees of lesions (p=0.085>0.05). It is possible that the increased expression of IL-1α, both in the dermis and epidermis, will not only contribute to comedogenesis but may also lead to the initiation of non-specific sub-clinical inflammation such as papulopustular and nodular. Acne vulgaris results in the appearance of comedones, papules, pustules, nodules and acne scars.[11]

Comedones and micro-comedones are non-inflammatory lesions of acne vulgaris.[12] Papules and pustules can also occur due to inflammation, resulting in clinical signs of redness and edema which eventually enlarge to form a nodule.[13] AV lesions can be non-inflammatory lesions in the form of comedones (closed or open) and inflammatory lesions in the form of papules, pustules, nodules and/or cysts.[1]

The results of this study on comedonal, papulopustular and nodular AV lesions showed the sampe strong IL-1α expression, this indicates that Interleukin-1 plays a central role in the regulation of inflammation and immune response in acne vulgaris.[14]

High levels of IL-1α have been proven playing a role in acne formation in the presence of proinflammatory cytokines that have been shown to trigger hyperkeratinization on the outside of the sebaceous duct wall (under the funnel) in vitro.

Conclusion

There was no significant difference in the expression of IL-1α that appeared in various degrees of acne vulgaris lesions

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