Original Article

Levels Of The Proinflammatory Cytokines: Interleukin-8, Interleukin-12, Tumor Necrosis Factor-α In Severe Acne At Makassar

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ABSTRACT

Background : The etiology of acne vulgaris (AV) is uncertain, in general, it is a multifactorial disease. Propionibacterium acnes induces toll-like receptor-2 (TLR-2) which causes the release of proinflammatory cytokines such as Interleukin-8 (IL-8), Interleukin-12 (IL-12) and Tumor Necrosis Factor (TNF-α). This mechanism leads to inflammation and tissue damage in AV that will affect the incidence of severe AV. Methods : This study aims to know the levels of IL-8, IL-12, and TNF-α in AV patients with severe lesions using a descriptive study. Results : 83 patients with severe AV obtained varying levels. Levels of IL-8 varied between 25.32 -64,783.43 pg / ml with an average (mean) ± 11035.00 14768.00 pg / ml, IL-12 levels varied between 17.98 to 61.33 pg / ml with a mean of 24.48 ± 6.84 pg / ml, and TNF-α levels varied between 13.18 to 239.84 pg / ml with a mean of 158.73 ± 52.32 pg / ml. Conclusion : The result of descriptive analysis from the three proinflammatory cytokines was that interleukin-8 has the highest levels in AV patients with severe pustules lesions.

1. Introduction

Acne vulgaris (AV) is a chronic inflammatory disease characterized by pilosebaceous follicle comedos, papules, pustules, nodules and scarring. Comedos are the early sign of AV lesions, while the papules and pustules are the result of inflammation thus producing erythema and edema that may be enlarged to form nodules. Acne vulgaris is usually found on the face, chest, back and upper arms.

Acne vulgaris is often discovered by dermatologists, especially in adolescence and usually continues to adulthood with a lifespan of 11-30 years, while the peak incidence at the age of 18 years. In the United States 40-50 million people suffered from AV each year and the number of visits at the dermatologist account for 20% of all visits. Some studies suggest that the prevalence of AV in some countries reaches up to 70-87%. Based on reports from patient visits at the Dermatovenereology Department of Ciptomangunkusumohospital Jakarta, the number of AV patients visits in 2010 were 2498 visits with new cases reaches up to 756 patients (30.37%). The data from medical records in the Dermatovenereology Department of Wahidin Sudirohusodo Hospital Makassar revealed number of patients visit with AV-nodular pustules in 2012 were 31 patients (19.53% of all AV patients visit).

The etiology of AV is uncertain. However, in general, it is a multifactorial disease characterized by follicular hyperkeratinization, increased activity of the sebaceous glands, hypercollonization of P. acnes, inflammation and genetic.

Propionibacterium acnes is a microaerophilic organism in acne lesions. Although it has not been proven in early lesions such as microcomedo, but their existence in some lesions is almost certainly. Propionibacterium acnes promote inflammation through a variety of mechanisms and stimulate inflammation by producing proinflammatory mediators that spread through the follicle walls.

Propionibacterium acnes induces tolllike receptor-2 (TLR-2) on polymorphonuclear monocytes cells (PMN) surrounding the sebaceous follicles to release proinflammatory cytokines such as IL-1, IL-8, IL-12 and TNF-α. This mechanism leads to inflammation and tissue damage in AV so it will affect the incidence of severe AV. Painful nodules is the severe inflammatory response in AV, which can lead to psychosocial problems for the patients.

Activation of TLR - 2 due to P. acnes action on monosit, causing the released of cytokines IL-12 and toll-like receptors (TLRs) and other inflammatory cells may be involved in the pathogenesis of acne. Given this data, TLR-2 seems to be the target of therapeutic intervention for inhibitory inflammatory cytokine response in AV and other inflammatory conditions. Treatment with all-trans retinoic acid (ATRA) can downregulates TLR-2 and reduces P. acnes-induced inflammatory cytokines, including IL-12 and TNF-α. In vitro, zinc can also inhibit the expression of TLR-2
by keratinocytes. Some study report, IL-12 may play an important role in treating various diseases such as viral infections, bacterial and cancer.

Research on IL-8 have been reported by Ali et al who were investigating the expression of IL-8 in skin biopsies from inflammatory lesions compared with normal AV and found that the expression of IL-8 in the lesions of AV is higher than the normal skin samples (p < 0.001). The production of the IL-12 cytokine is one of the major proinflammatory cytokines produced by monocytes in response to gram positive organisms. Excess production of IL-12 found in several inflammatory diseases involving tissue damage. Heymann reported that P.acnes were either induced production of IL-12 through TLR-2. In the study by Sugisaki et al, it was also reported an increasing in IL-12p40 in the blood of patients with AV were higher than in normal controls.

Based on the above facts, further researches are needed to investigate the levels of IL-8, IL-12, and TNF-α in patients with AV lesions in Makassar.

2. Materials and Methods

This study was an observational study using descriptive design, namely variable study measured at the same time period. This research was conducted at the outpatient clinic of Dermatovenereology department in Dr. Wahidin Sudirohusodo hospital, and Department of Microbiology, Faculty of Medicine Hasanuddin University from July-December 2013.

The study population was all patients with severe AV who were visiting the outpatient clinic of Dermatovenereology Department, Dr. Wahidin Sudirohusodo hospital.

The samples were all members of the population who came to the outpatient clinic of the Dermatovenereology Department, Dr. Wahidin Sudirohusodo hospital at the time of the study and met the following criteria:

- The inclusion criteria are patients with severe AV based on Combined Acne Severity Classification criteria were assessed by one person dermatologist and sex, patients with severe AV who does not suffer from other infectious diseases and patients with severe AV who were available to participate in the study and asked for consent in writing (signed informed consent) after getting enough information about the advantages and undesirable things that may occur during the study.

- The exclusion criteria are AV patients with severe systemic disease, patients who received treatment of severe AV with retinoids, antibiotics and anti-inflammatory agents during the last 1 month, patients with severe AV who were using hormonal contraception and severe AV patients with pregnancy and lactation.

Sample calculation is performed using Izaac Michael tables with α = 5% and N = 110 with n = 83 samples found.

Study procedures

- Patients with severe AV who were visiting the outpatient clinic of Dermatovenereology Department, Dr. Wahidin Sudirohusodo hospital that meet the inclusion criteria will be investigated for history, physical examination, and was asked to join the study after signing an informed consent.

b. A physical examination is done to diagnose severe AV. At the same time the patients picture was also being taken from the front, left and right side with a digital camera and shooting distance of 20 cm.

c. Specimens were taken from the pustules of inflammatory lesions. The lesions were previously cleansed with 70% alcohol at that location, then the pustules roof were pierced meticulously with the tip of a sterile 27G needle without causing bleeding. Pus was then taken using blackhead extractor, partly laid pussy appendor tube which already containing PBS. After such treatment, topical antibiotic was applied on lesions.

d. Quantification of cytokines

IL-8, IL-12 and TNF-α were calculated by ELISA (Enzyme-Linked Immunosorbent Assay) using Human Quantikine® kit (R&D SYSTEMS). The examination was conducted within 36 hours after the elution process. Prepare all reagents, add 100 mL assay diluent to each sample to each well, incubated for 2 hours at room temperature. Aspiration and rinse 4 times. Adding 200 uL of conjugate to each well, incubated for 2 hours at room temperature. Aspiration and rinse 4 times. Adding 200 mL of substrate solution to each well, incubated for 30 min at room temperature. Adding 50 mL stop solution to each well. Detecting the optical density in each well within 30 minutes using an microplate reader with a wavelength of 540 nm.

3. Results and Discussion

The number of samples in this study were 83 people consisting of 30 men (36.1%) and 53 women (63.9%).

Samples in this study consisted of one person (1.2%) with low education, 38 (45.8%) secondary education people, and 44 (53%) are highly educated. The samples consisted of 13 people (15.7%) of employees, 3 (3.6%), housewives, and 67 (80.7%) of students. Most of the samples are Buginese (41%) and Makassarneese (34.9%).

Descriptive analysis of age and long-suffering of AV revealed that of 83 people with severe AV, patients age varied between 13-45 years with a mean of 20.8 ± 5.4 years, while the long-suffering of AV varies between 1-120 months with a mean of 27 ± 22.9 months.

The results of levels measurement of interleukin-8, interleukin-12 and TNF-α are shown in Table 1.

| Table 1. Descriptive analysis result of interleukin-8, interleukin-12 and TNF-α levels in severe AV |
|---|---|---|---|---|---|
| Level | n | Minimum | Maximum | Mean | SD |
| IL-8 | 83 | 17.98 | 61.33 | 24.48 | 6.84 |
| IL-12 | 83 | 25.32 | 64783.43 | 11035.0 | 14768.00 |
| TNF-α | 83 | 13.18 | 239.84 | 158.73 | 52.32 |
From Table 1 showed that out of 83 people with severe AV, levels of IL-8 varied between 25.32-64783.43 pg/ml with an average (mean) ± 11035.00 14768.00 pg/ml, the levels of IL-12 varied between 17.98 to 61.33 pg/ml with a mean of 24.48 ± 6.84 pg/ml, and TNF-α levels varied between 13.18 to 239.84 pg/ml with a mean of 158.73 ± 52.32 pg/ml.

4. Discussion

An inflammatory response to tissue damage in the acute phase is characterized by increased blood flow, vascular permeability, fluid accumulation, and leucocyte and inflammatory mediators such as cytokines. Subacute/chronic phase is characterized by the development of humoral and cellular immune responses specific to the damaged area. During the acute and chronic phases, various dissolvable factors are involved in leucocyte recruitment through increased expression of cellular adhesion molecules and chemotaxation. The dissolved factors that mediate the response were divided into four main categories: 1) Inflammatory lipid metabolites such as platelet activating factor (PAF) and a number of arachidonatidic derivatives (prostaglandins, leukotrienes, lipoxins) derived from cellular phospholipids; 2) three lines of protease substrate that produces a number of pro-inflammatory peptide; 3) nitric oxide, a potent endogenous vasodilator, which plays a role in the inflammatory process; and 4) a group of cells that produce polypeptide, known as cytokines. Most cytokines are multi-functional. Cytokines are involved in the synergistic and antagonistic interaction network and express the negative and positive effects on various cellular targets.

Proinflammatory cytokines can be divided into two groups: those who are involved in acute inflammation and are responsible for the chronic inflammation. Some cytokines that play a role in mediating acute inflammatory reaction are IL-1, TNF-α, IL-6, IL-8, IL-11 and other cytokines, G-CSF and GM-CSF. Among these cytokines, IL-1 and TNF-α are the most potent inflammatory molecules. Both are primary cytokines that mediate acute inflammation induced by lipopolysaccharide of bacteria. Besides a role in mediating acute inflammatory reactions, IL-1 and TNF-α also mediate the chronic inflammatory process with their contribution to cellular inflammation, while IL-8 only mediates acute inflammatory reaction.

From measurements of cytokine levels in pustules lesions of patients with severe AV in this study, it appears that IL-8 have the highest levels (mean ± 14768.00 11035.00 pg/ml) compared with the levels of TNF-α (mean 158.73 ± 52.32 pg/ml) and the levels of IL-12 (mean 24.48 ± 6.84 pg/ml). It consistent with study by Vowels et al, who examined the production of proinflammatory cytokines interleukin-8, IL-1β, and TNF-α by human monocytes. Incubation of P.acnes with human monocyte cells also produce IL-8 production.

Acute inflammation is characterized by infiltration of neutrophils at the sites of inflammation. Local production of chemotactic factors mediates the sequence of inflammatory infiltration in the area. Interleukin-8 is a cytokine that plays a role in the activation of chemotactic of neutrophils. This could be the explanation for the levels of IL-8 were higher in AV patients with pustules lesions.

Interleukin-8 is a human chemokine prototype in the form of a polypeptide of about 8-10 kDa which were used for the basic process, binding of heparin inflammation and tissue repair. Characteristics of IL-8 were found in the two residues of Cysteine near the N-terminus which is separated by an amino acid. Unlike general cytokine, IL-8 is not a glycoprotein. Interleukin-8 is produced by a variety of cells, including monocytes, neutrophils, T cells, fibroblasts, endothelial cells and epithelial cells, after exposure to an antigen or inflammatory stimuli (ischemia and trauma). Two forms of IL-8 (77 and 72 CXC CXC) are secreted by neutrophils when activated. Production of IL-8 has always been associated with excessive inflammatory diseases, such as asthma, leprosy, psoriasis, pregnant and lactating mothers. Interleukin-8 can also induce tumor development as one of its angiogenic effect, in addition to vascularization. Of several chemokines that trigger chemotaxis of neutrophils, IL-8 is a strong chemotactic agent. Shortly after triggered, neutrophils become activated and undergo shape changing due to the activation of integrins and the cytoskeleton actin. Basophil, T cells, monocytes and eosinophils also show chemotactic response to IL-8 by triggering integrin activation required for adhesion to endothelial cells during migration.

Among the proinflammatory mediators, IL-8 was identified as neutrophil activating peptide in conjunction with Paceswhichinduces chemotactic factors that play a role in attracting neutrophils to the pilosebaceous unit. Production of IL-8 by P.acnes is through NF-kappa B. Research by Ghoname et al showed increased expression of IL-8 significantly in inflammatory lesions compared to the skin without lesions AV. This is consistent with findings of some researchers that states that activation of TLR-2 on monocytes could release cytokines such as IL-12 and IL-8.

All and colleagues who were investigating the expression of IL-8 in skin biopsies from inflammatory lesions compared with normal AV found that the expression of IL-8 in the lesions of AV is higher than the normal skin samples (p < 0.001). Sugisaki et al, stated that the production of IL-8 in the blood of patients with AV then stimulated with Paces, is higher than the non-AV control. Production of IL-12 cytokines by clicking macrophages are essential in the induction of Th1 cells during the early immune response to pathogens and is one of the major proinflammatory cytokines produced by monocytes in response to Gram-positive organisms. Some studies suggest that IL-12 may play an important role in treating various diseases such as viral infections, bacterial and cancer.

Th1 cells produce IFN-γ and IL-2 that enhance macrophage activation and maturation of cytolytic T cells, resulting in cell-mediated response that is effective against intracellular pathogens. Macrophages are a major source of IL-12 production in response to intracellular particular pathogen, such as Listeria monocytogenes. More recently, Chehimi et al reported decreased production of IL-12 by macrophages in Human Immunodeficiency Virus infected individuals and can contribute to reduce the Cell-Mediated Immunity seen in AIDS.

Kim et al conducted a study with a sample of normal human monocytes and stimulated it with various dilutions sonicate of Paces, and cytokine production was measured and found that Paces were able to induce IL-12 production through TLR-2 pathway. Study by Sugisaki et al, also reported an increasing in IL-12p40 in the blood of patients with AV higher than normal controls.

Tumor necrosis factor-α is one of the proinflammatory cytokines that induce strong inflammatory response and a key regulator in innate immunity. Tumor necrosis factor-α increased the expression of major histocompatibility complex (MHC) class II
on activated T cells and is a cofactor of B cell proliferation and immunoglobulin production. Factors that influence the production of TNF-α would affect the degree of inflammation that relate to the severity of AV.

Conclusion

A descriptive analysis, interleukin-8 levels are higher (mean ± 14768.00 ± 11035.00 pg/ml) compared with the levels of TNF-α (mean 158.73 ± 52.32 pg/ml) and the levels of IL-12 (mean 24.48 ± 6.84 pg/ml) in AV patients with severe pustules lesions.

Bibliography


