



Contribution of Propionibacterium Acnes Bacteria in Patients with Acne Vulgaris in Makassar

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Abstract

Propionibacterium acnes is a microaerophilic organism in acne lesion. The study aims to determine the relationship between the number of Propionibacterium acnes bacteria colonies and the degrees of acne vulgaris. The research was conducted at high school in Makassar city on July 2017- August 2017 as an observational analytic study with cross sectional method. The samples were forty-four (44) acne vulgaris patients of 14 to 20 years old at high schools in Makassar city. Blackheads were taken from acne vulgaris lesions of the 44 sample. The process was followed with gram stain, anaerobic bacterial culture, and polymerase chain reaction examination (PCR). The Results from the anaerobic bacteria culture examination of 44 people were, 33 (75%) Staphylococcus epidermididis, 1 (2,3%) Staphylococcus xylosus, 4 (9%) Staphylococcus hominis, 1 (2,3%) Staphylococcus aureus, 1 (2.3%) Staphylococcus haemolyticus, 1 (2.3%) Staphylococcus capitis, 2 (4,5%) Staphylococcus warneri, 1 (2.3%) Lactobacillus plantarum and none of propionibacterium acnes. Furthermore the PCR examination showed that there was no Propionibacterium acnes on mild acne vulgaris but in five students (17,8%) had positive results of Propionibacterium acnes in moderate and severe acne. It showed that there was a statistical significance result with biological relevance, Propionibacterium acnes contribute to moderate and severe acne.

Keywords: Anaerobic culture; PCR; Propionibacterium acne.

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1. Introduction

Acne vulgaris is a chronic inflammatory skin disease known by the presence of open comedones (blackheads) and closed comedo (whiteheads) and inflammatory lesions including papules, pustules, nodules or cysts. Acne generally occurs in young adults about 85% of that age group and can survive to adulthood [1]. The most common clinical features are; blackheads, papules and pustules and 1: 4 cases have scars [2].

Acne is formed on hair follicles in the skin, such as cells accumulated in the skin surface and acne also buried in the follicle. Improvement in the accumulation process along with the cohesive increase of these cells (hyperkeratotic retention) and increased production of sebum cause comedone formations (clogged hair follicles containing sebum, keratin and normal flora), consider the following process and begin the inflammation. The main bacteria known as *Propionibacterium acnes* naturally occur deep within the follicle, these bacteria are actually non-pathogenic but contribute to the development of acne, bacteria accumulation and release the proinflammatory mediator that causes the formation of papules and pustules that aggravate the severity of the disease. In case of papules or pustules can develop into nodules or cysts [3].

Propionibacterium acnes is a microaerophilic organism in acne lesions. Although it has not been proven to be present in early lesions of microculture, its presence in some lesions is almost certain. *Propionibacterium acnes* promotes inflammation through various mechanisms and stimulates inflammation by producing proinflammatory mediators that spread through the follicle wall. *Propionibacterium acnes*, previously classified as *Corynebacterium parvum*, has been implicated in the pathogenesis of acne vulgaris for more than 100 years. First, *P. Acne* was initially introduced in early 1896, these microorganisms were found in acne vulgaris lesions and recommended as the main cause of acne and subsequent research supported the theory. Other studies provide evidence that *Propionibacterium acnes* causes inflammation of acne vulgaris. It demonstrated that *Propionibacterium acnes* injected in sterile keratin cysts and inflamed and ruptured, another study of *propionibacterium acnes* significantly increased in pilosebaceous gland especially in patient with acne vulgaris. The debate about *Propionibacterium acnes* in the pathogenesis of acne appeared in the early 1960s, when a study showed that *Propionibacterium acnes* was also on the surface of healthy human skin. The amount of *Propionibacterium acnes* between acne and control patients did not correlate the severity of acne vulgaris patients and some inflamed lesions did not contain the bacteria. Further evidence to support the hypothesis that *Propionibacterium acnes* is involved in the pathogenesis of acne vulgaris by providing antibiotics such as erythromycin and clindamycin showing clinical improvement. Recent studies concluded although *Propionibacterium acnes* is directly involved in acne vulgaris but a few evidence demonstrated the mechanism of *Propionibacterium acnes* contributes to the pathogenesis of acne vulgaris [4].

The involvement of *Propionibacterium acnes* in the pathogenesis of acne is still controversial. However, there is controversy over *Propionibacterium acnes* as a normal flora of healthy human skin and an exclusive normal bacteria on the face. Many in vitro and in vivo studies showed that *Propionibacterium acnes* induces an inflammatory response within the skin's parenchymal cells and cellular immune system. The previous research by Kwon & Suh [5], reported that in certain sub-populations *Propionibacterium acnes* is responsible for the pathogenesis of acne vulgaris and other bacterial species only as observers.

Other evidence in vitro, indicating the various ways in which *Propionibacterium acnes* may be involved in inflammation, suggests the possible presence of this organism in acne inflammation [6]. *P. acnes* is not infectious but it has a role in the occurrence of acne, which is important in the microenvironment of the follicle in bacterial colonization into the follicle ducts and causing the production of inflammation. There is an association between *P. acnes* reduction and clinical improvement of acne. This reduction in *P. acnes* is associated with a reduction in proinflammatory mediators. The host's response to the inflammatory stimulus seems to explain the variation in the intensity of inflammation.

Based on the above background, the researchers are interested in conducting research. The aim of this study is determine the relationship of the colonies of *Propionibacterium acnes* with degrees of acne vulgaris.

2. Material and Methods

2.1 Time and Place

Location and Time of this study was conducted in Dermatology and Venereal disease of Hasanuddin University Hospital, High School in Makassar City, The Microbiology laboratory of Hasanuddin university Hospital, Makassar Health Laboratory which was held from July to August 2017.

2.2 Design Study and Variable

This research is an observational research using cross sectional design. The research variables consist of independent variable (number of bacterial colonies), dependent variable (Acne Vulgaris), and confounding variables (Examination Culture of *propionibacterium acnes* bacteria with blood agar media and PCR examination).

2.3 Population and Sample

In the Study, the classifications were mild, moderate, severe stage of acne in high school in Makassar. The samples of this study were the entire affordable populations met the inclusion and exclusion criteria.

2.4 The Method

The patients with mild, moderate, high school acne vulgaris in Makassar met the inclusion and exclusion criteria for anamnesis, physical examination, and were asked to be involved in the study after signing informed consent. Physical examination is performed to confirm the diagnosis of mild, moderate, and severe Acne Vulgaris. Furthermore, Gram Examination, Culture of *Propionibacterium Acnes* Bacteria, and PCR Examination. The data obtained will then be analyzed and reported as research results.

2.5 Data Analysis

Data in this research will be processed with the help of computer using SPSS version 18. Data processing is analyzed with Chi square. All results of the analysis will be presented in tabular form with annotations.

3. Results

This study conducted by observational and cross sectional design to investigate the relationship of the colony of *Propionibacterium acnes* bacteria with degree of acne vulgaris. The research was conducted at Dermatology and Venereal Disease polyclinic in Hasanuddin University Hospital, High School School in Makassar, Microbiology laboratory of Hasanuddin University Hospital and Makassar Health Laboratory which was held from July to August 2017. Samples of 44 people with acne vulgaris who meet the criteria of high school in makassar city.

Based on the characteristics of the samples, 30 samples were obtained un this study and the male sample was 34 peoples (56.7%) and women was 10 peoples (43.3%). The 15-16 age group was the highest frequency group with 35 peoples (79.5%), while the lowest was 9 samples in the 17-18 years age group (20.5%). Based on family history, 25 peoples (56.8%) have family history and 19 persons (43.2%) without family history (attachment, Table 1).

Table 1: The characteristics of Acne vulgaris patients in Study

| Category | Frequency (n) | Percentage (%) |
|-----------------|---------------|----------------|
| Sex | | |
| Male | 34 | 77,3 |
| Women | 10 | 22.7 |
| Age | | |
| 15-16 y.o | 35 | 79.5 |
| 17-18 y.o | 9 | 20.5 |
| Education | | |
| High School | 44 | 100 |
| Family history | | |
| Yes | 25 | 56.8 |
| None | 19 | 43.2 |
| Food | | |
| Milk | 7 | 15.9 |
| Chocolate | 3 | 6.8 |
| Oily food | 32 | 72.7 |
| High Sugar food | 2 | 4.6 |
| Total | 44 | 100 |

Anaerobic bacteria examination of 44 people found 33 (75%) *Staphylococcus epidermididis*, 1 (2.3%) *Staphylococcus xylosus*, 4 (9%) *Staphylococcus hominis*, 1 (2.3%), *Staphylococcus aureus*, 1 (2,3 %) *Staphylococcus haemolyticus*, 1 (2.3%) *Staphylococcus capitis*, 2 (4.5%) *Staphylococcus warneri*, 1 (2.3%) *Lactobacillus plantarum* and no *propionibacterium acnes* (attachment, Table 2).

Table 2: The results of Anaerobic Culture Examination in comedo lesion

| Microba | TheAnaerob Culture result | Total (%) |
|-------------------------------------|------------------------------|-------------|
| <i>Propionibacterium Acnes</i> | 0 | 0 |
| <i>Staphylococcus epidermididis</i> | 33 | 75 |
| <i>Staphylococcus xylosus</i> | 1 | 2.3 |
| <i>Staphylococcus hominis</i> | 4 | 9 |
| <i>Staphylococcus aureus</i> | 1 | 2.3 |
| <i>Staphylococcus haemolyticus</i> | 1 | 2.3 |
| <i>Staphylococcus capitis</i> | 1 | 2.3 |
| <i>Staphylococcus warneri</i> , | 2 | 4.5 |
| <i>Lactobacillus plantarum</i> | 1 | 2.3 |
| Total | 44 | 100 |

Acne was found in 16 peoples (36.4%) mild acne, 14 peoples (31.8%) moderate acne, 14 peoples (31.8%) severe acne.

While on the result of PCR *Propionibacterium acnes* examination from 44 people the frequency was found *Propionibacterium acnes* of 5 or 11.4% while negative result found 39 or 88.6% (appendix, Table 3). Positive PCR results in AD1, AD13, NR4, NR14, NR15. Where appropriate with positive control values and tire target was 344 bp (attachment, Figure 1).

Table 3: The Differences Results of PCR on Mild, severe and moderate *Acne vulgaris*

| | PCR | | TOTAL |
|---------------|----------|----------|-------|
| | POSITIVE | NEGATIVE | |
| Mild acne | 0 | 16 | 16 |
| Count % total | 0% | 36.4% | 36.4% |
| Moderate acne | 3 | 11 | 14 |
| Count % total | 6.8% | 25.0% | 31.8% |
| Severe acne | 2 | 12 | 14 |
| Count% total | 4.5% | 27.8% | 31.8% |
| Total | 5 | 39 | 44 |
| Count % total | 11.4% | 88.6% | 100% |

Information : PCR = *Polymerase chain reaction*

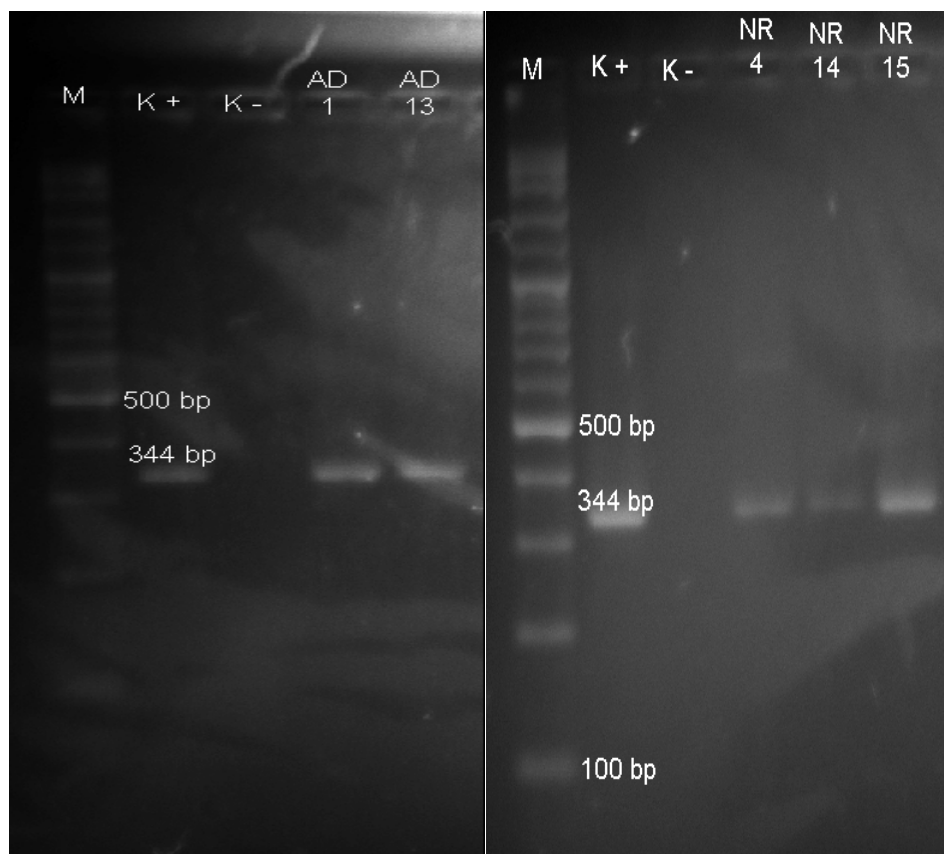


Figure 1: PCR Examination : Positive result in patients with Acne Vulgaris

PCR results were not found *Propionibacterium acnes* in mild acne vulgaris, but in moderate and severe acne found 5 positive *Propionibacterium acnes* (17.8%). The conclusion was statistically significance and biological relevance, *Propionibacterium acnes* has contributed to moderate and severe acne.

4. Discussion

This study showed that anaerobic bacteria examination of 44 people found 33 (75%) *Staphylococcus epidermidis*, 1 (2.3%) *Staphylococcus xylosus*, 4 (9%) *Staphylococcus hominis*, 1 (2.3%), *Staphylococcus aureus*, 1 (2.3%) *Staphylococcus haemolyticus*, 1 (2.3%) *Staphylococcus capitis*, 2 (4.5%) *Staphylococcus warneri*, 1 (2.3%) *Lactobacillus plantarum* and no *propionibacterium acnes* and PCR results not found *Propionibacterium acnes* on mild acne vulgaris, but in moderate and severe acne found 5 positive *Propionibacterium acnes* (17.8%), so statistically significant of *Propionibacterium acnes* has contributed to moderate and severe acne.

Characteristics of the sample in this study showed 44 patients with mild, moderate and severe acne vulgaris included in the inclusion criteria, found the age distribution of 15-17 years were 43 people, 18 years age group was 1 person. All age groups may be affected, but acne is a more common disorder in adolescence. Early acne lesions begin to appear at the age of 8-9 years group and approximately 50-60% are present in adolescents. The prevalence of 80-85% acne vulgaris in adolescents with peak incidence was 15-18 years, 12% in women aged >

25 years and 3% at 35-44 years. The prevalence of acne vulgaris sufferers aged 15-25 years about 58%, the study of patients with acne vulgaris most at the age of 18-25 years. most people with acne vulgaris at age 12-39 years [7].

Patients who have daily oily habits were 32 peoples, who have a habit of drinking milk every day were 7 people, who have a habit of eating chocolate were 3 peoples and who have a habit of sweet foods (high sugar) were 2 peoples. Diets that can induce hyperinsulinemia, foods that contain high glycemic levels can lead to Androgen responses that may aggravate acne vulgaris. Although this diet is considered an agent induced acne, this diet method is a contributing factor to acute and chronic hyperinsulinemia. Recent evidence suggests that hormones triggered by food hyperinsulinemia will trigger an endocrine response that simultaneously does not regulate tissue growth and promote androgen synthesis. Various studies of chocolate on the appearance of acne and concluded that the absence of proof that chocolate affects the formation of acne [8].

Family history with acne, especially in fathers and mothers, increases the risk of acne in their children. Several studies have shown that genetic factors play a role in the susceptibility of acne. Research in Germany shows that 45% of acne occurs in boys who have a history of one or both parents suffering from acne and 8% with no history of acne in the elderly. It is known that the number, size and activity of the sebaceous glands are passed down from generation to generation. And based on epidemiological studies conducted in schools in France found among 913 adolescents aged 11-18 years with a history of acne in the father obtained 16% results in the acne group compared to 8% in the group without acne lesions. Meanwhile, a history of maternal acne was also studied and 25% in the acne group and 14% in the group without acne lesions. In the history of acne studied in siblings, there was a 68% gain in the acne group and 57% in the group without acne lesions. In addition, a history of acne in the father or mother is often associated with severe acne events or acne does not respond to treatment.

Patients with mild, moderate and severe acne vulgaris who have examined anaerobic and PCR culture, which amounted to 44 people, the results were found in the anaerobic culture results not found *Propionibacterium* acnes. The culture examination was performed in accordance with the anaerobic bacterial anchovy procedure by taking the blackhead lesion by using a comedone extractor on acne vulgaris and cultured on blood agar medium in an anaerobic atmosphere then incubated at 37 ° C for 24-48 hours. While on the PCR examination there is no significant relationship on the contribution of *Propionibacterium* acnes in patients with acne vulgaris PCR examination. Where *Propionibacterium* acnes was found in 3 (6.8%) patients with moderate acne and 2 (4.6%) severe acne patients. Polymerase Chain Reaction is an enzymatic method for DNA amplification by in vitro method.

Anaerobic culture examination results were not found *Propionibacterium* acnes. The role of *Propionibacterium* acnes in acne vulgaris remains a controversial topic according to Shaheen & Gonzales [6], based on microbiology derived from the comedone culture *Propionibacterium* Acnes may not be involved in the initiation of this lesion, but there are also data suggesting the possible role of pathogenesis *Propionibacterium* acnes in the formation of comedogenesis . To resolve the *Propionibacterium* acnes controversy, microbiological research from 59 blackheads lesions (29 open comedones and 30 closed comedo) was isolated from microdissection and

skin biopsy. Only About 55% and 22% found colonization of *Propionibacterium acnes* and *Staphylococcus* in blackheads lesions as well as *ptyrosporum* sp about 74% of the lesions. Comparing the findings of their study on microbial examination there was no significant difference in colonization of *Propionibacterium acnes* in normal follicles and acne vulgaris [6].

The Species of epidermidis *staphylococcus* bacteria were found in the most cases, 33 cases (75%). Bacteria known as *Staphylococcus epidermidis* are naturally present in the follicle, these bacteria are not pathogenic but contribute to the development of acne, bacteria accumulate and release the proinflammatory mediator that causes the formation of papules and pustules that aggravate the severity of the disease [3]. *Staphylococcus epidermidis* is a facultative bacteria, and it has been reported that the bacteria can ferment in aerobic conditions. *Staphylococcus epidermidis* can control the overgrowth of *Propionibacterium acnes* through the fermentation process. The clinical trial showed that anti *Propionibacterium acnes* on the fermentation activity of *staphylococcus epidermidis*, colon *Staphylococcus epidermidis* (10^5 CFU) redistributed to glycerol containing medium *propionibacterium acnes* medium (10^7 CFU) then visible on the *propionibacterium acnes* colony growth plate is inhibited, indicate that the glycerol fermentation process in *staphylococcus epidermidis* can reduce the growth of *Propionibacterium acnes* [9]. The Current report results show that the evidence based source provide the interaction between *Propionibacterium acnes* and *staphylococcus epidermidis* and the results of these interactions vary according to body areas such as the cheeks and forehead [10].

5. Conclusion

The authors concluded that there was no association between *Propionibacterium acnes* with the severity of acne vulgaris. *Propionibacterium acnes* contributes to acne vulgaris in PCR examination. Anaerobic cultures were not found in *Propionibacterium acnes* and other types of bacteria were *Staphylococcus epidermidis*, *Staphylococcus xylosum*, *Staphylococcus hominis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus warneri*, *Lactobacillus plantarum*. The researchers suggested that a study comparing male and female gender samples in acne vulgaris patients on examination of the bacterial species *Propionibacterium acnes* using anaerobic bacteria, PCR, culture examination and the retrieval technique of all lesions of the acne vulgaris sample was preferred in closed comedone lesions.

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6. Competing Interest

The authors declare that they have no competing interests.

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