Immunohistochemical expression of interleukin 1 beta in papule biopsies from patients with acne vulgaris

Le Thai Van Thanh,1,2 Le Vi Anh,1 Tran Huong Giang,1 Ta Quoc Hung,1 Van The Trung,2 Nguyen Lam Vuong1
1Department of Dermatology and Skin Aesthetics, University Medical Center at Ho Chi Minh City; 2Department of Dermatology and Venerology, Faculty of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City; 3Department of Pathology, Faculty of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City; 4Department of Medical Statistics and Informatics, Faculty of Public Health, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam

Abstract

Acne vulgaris is the most common inflammatory disease of the skin. IL-1β has been found in acne lesions and is a promising target for therapy, but the evidence is limited. Therefore, this study was conducted to investigate the immunohistochemical expression of IL-1β in papule biopsies of inflammatory acne and its association with disease severity. This study involved 20 patients with acne vulgaris (13 females, median age: 22 years). Samples were taken using punch biopsy. Immunohistochemical IL-1β expression was semi-quantitatively assessed as absent, mild, moderate or strong. Disease severity was evaluated according to the Global Acne Grading System (GAGS). There were 7 patients with mild disease and 11 patients with moderate disease. Median GAGS score was 20. Mild and moderate accounted for 65% and 30% for dermal IL-1β expression, 60% and 40% for epidermal expression, and 70% and 15% for perifollicular expression. Moderate-strong perifollicular expression had significant higher GAGS score than absent-mild expression (median: 22 versus 16). This study shows the elevated immunoreactivity of IL-1β in papule biopsies of inflammatory acne vulgaris. The levels of IL-1β expression also correlates with disease severity. IL-1β could be a good candidate for targeting treatment of acne vulgaris.

Introduction

Acne vulgaris is the most common inflammatory disease of the skin which affects to 80% of the population at least once in their lifetime. The main causes of acne are abnormal follicular keratinization, excessive sebum secretion, inflammatory processes, and the presence of the anaerobic bacterium Propionibacterium acnes (P. acnes), which lead to different types of skin lesions. Deep lesions can cause relevant complications such as scarring, they affect patient’s self-confidence and sometimes cause depression. Inflammation has been identified as an important factor in the pathogenesis of acne and P. acnes plays an important role in the development of inflammatory lesions. This bacterium can stimulate keratinocytes and monocytes to produce interleukin (IL)-6, IL-8, IL-1β, tumor necrosis factor (TNF)-alpha, IL-8 and IL-12. Patients with severe lesions (e.g., nodules, cysts) require a long-term treatment and may have drug resistance or side effects such as skin irritation, increased liver enzymes and dyslipidemia, but the disease can still relapse. Currently, more studies on the pathogenesis are conducted in order to generate therapeutic targets in inflammatory acne.

Among the proinflammatory mediators, IL-1β has been found in inflammatory acne lesions under many active forms. The formation of IL-1β depends on the activation of inflammatory complexes NLRP3 from monocytes that contact with P. acnes bacteria. It has been shown that the inflammatory reactions caused by P. acnes can be prevented by selectively targeting the inflammatory complexes or IL-1β. Several studies have shown potential therapeutic effect of drugs on IL-1β in patients with autoimmune syndromes, including acne as a symptom. IL-1β is a promising target in acne vulgaris that needs to be investigated in order to understand the disease pathogenesis and create novel therapeutic agents for acne. Therefore, this study was conducted to investigate the immunohistochemical (IHC) expression of IL-1β in skin biopsies of inflammatory acne and its association with disease severity and histopathological changes. The study can help to better understand the role IL-1β in the pathogenesis of acne and provide knowledge for targeted treatments in inflammatory acne.

Materials and methods

Patients

This study involved 20 patients with acne vulgaris treated in the University Medical Center at Ho Chi Minh City (a tertiary hospital in Ho Chi Minh City, Vietnam) from November 2019 to July 2020. The study was in accordance with the Declaration of Helsinki and was approved.
by the institutional ethic committee before recruiting any patient. Inclusion criteria were: a) ≥ 18 years of age; b) a diagnosis of acne vulgaris; c) clinical manifestations of papules; d) receiving no treatment or stopping treatment for at least two months before the biopsy, and e) having no other inflammatory skin disease or previous autoinflammatory disease. We excluded patients who used immunosuppressive therapy within three months prior to biopsy and patients with a history of keloids or bad scarring. Collected variables included baseline characteristics of patients and acne, disease severity according to the Global Acne Grading System (GAGS),\(^\text{10}\) and the level of IL-1\(\beta\) expression in tissue samples. Written informed consent was obtained from all patients before the biopsies.

**Biopsies and pathological examination**

All patients underwent 2-mm punch biopsy under local anesthesia. After taken out, samples were immediately fixed in 10% buffered formalin and processed to paraffin blocks. The samples were then stained by hematoxylin and eosin (H&E) to evaluate histopathological changes by a pathologist. All evaluations were semi-quantitatively assessed as mild, moderate and severe, including epidermal hyperplasia, follicular hyperkeratosis, angiogenesis and the extent of dermal inflammatory response, and the level of inflammatory infiltrate in epidermis and dermis.

**Immunohistochemistry**

In this study, we use a rabbit polyclonal IgG antibody to IL-1\(\beta\). This antibody is raised against a recombinant 153 aa human IL-1\(\beta\) with the N-terminal amino acid at position alanine 117. All incubations were performed at room temperature. Formalin-fixed, paraffin-embedded tissue specimens were cut into thin slides and de-waxed with EZ Prep solution. After submerged in phosphate buffered saline, slides were incubated in Epitope Retrieval Cell Conditioning 1 liquid (Vetana Medical System, Inc., SF, CA, USA) in 40 minutes and washed twice, followed by the incubation for 60 minutes with antibody diluted at 1:100. Slides were stained with hematoxylin in 30 seconds and were semi-quantitatively evaluated by a pathologist as absent (negative), mild, moderate and strong. Figure 1 shows a case with moderate IL-1\(\beta\) expression in epidermis and dermis (Figure 1A) and a case with moderate IL-1\(\beta\) expression in dermis (Figure 1B).

**Statistical analysis**

All variables were summarized using counts and percentages for categorical variables and median and interquartile range (IQR) for continuous variables. The GAGS score was compared between the group of absent or mild IL-1\(\beta\) expression and the group of moderate to strong IL-1\(\beta\) expression by Mann Whitney-\(U\) test. All tests were two-sided and \(P\)-value of $<0.05$ was considered as statistically significant. Analyses were done using the statistical software R version 3.6.3.

| Table 1. Baseline characteristics.                                      |
|-----------------------------------------------------------------------|
| All patients (N=20)                                                   |
| Sex female, n (%)                                                     | 13 (65) |
| Age (years), median (IQR)                                            | 22 (20 – 24) |
| Age when symptoms onset (years), median (IQR)                        | 17 (15 – 20) |
| Duration of the disease before biopsy (months), median (IQR)         | 60 (21 – 87) |
| GAGS score, median (IQR)                                             | 20 (16 – 21) |
| Disease severity, n (%)                                              |
| Mild (GAGS score: 1-18)                                              | 9 (45) |
| Moderate (GAGS score: 19-30)                                         | 11 (55) |
| Type of lesion, n (%)                                                |
| Papule                                                               | 20 (100) |
| Closed comedones                                                     | 20 (100) |
| Open comedones                                                       | 19 (95) |
| Pustule                                                              | 8 (40) |
| Nodule                                                               | 2 (10) |
| Cyst                                                                 | 0 (0) |

IQR: interquartile range; GAGS: Global Acne Grading System.

Figure 1. Moderate IL-1\(\beta\) expression. A) moderate IL-1\(\beta\) expression in epidermis and dermis; B) moderate IL-1\(\beta\) expression in dermis.
Results

Baseline characteristics of the study population are shown in Table 1. Females were predominant (65%). The median age of patients was 22 years (IQR: 20-24) and the median duration of acne before biopsy was 5 years. There were 9 patients with mild disease and 11 patients with moderate disease according to GAGS score (median GAGS score was 20). Papule and closed comedones were found in all patients and 95% had open comedones; while pustule and nodule occurred in 40% and 10% of the patients respectively.

With regard to pathological results, mild and severe epidermal hyperplasia accounted for 55% and 35% respectively. All cases had follicular hyperkeratosis (mild: 30%; moderate: 45%; severe: 25%) and angiogenesis and the extent of dermal inflammatory response (mild: 10%; moderate: 55%; severe: 35%). There were 70% of cases with absent inflammatory infiltrate in epidermis, however, all cases had inflammatory infiltrate in dermis (mild: 15%; moderate: 50%; severe: 35%). Dominant cells in epidermis were neutrophils (100%), while in dermis they were monocytes (Table 2).

IL-1β expression results are shown in Table 3. All cases had dermal and epidermal expression of IL-1β with most were mild or moderate. For dermal expression, mild and moderate accounted for 65% and 30%. For epidermal expression, mild and moderate accounted for 60% and 40% respectively. Regarding perifollicular expression of IL-1β, there were 10% with absent and the majority were mild (70%). The type of cells in dermal expression was monocyte (100%), endothelial cell (15%), fibroblast (5%) and giant cell (5%). Basal and squamous cells were found in epidermal expression and monocytes were found in perifollicular expression. Compare with absent-mild dermal and epidermal IL-1β expression, moderate-strong expression had a little higher median GAGS score, but the differences were not significant. However, the group of moderate-strong perifollicular IL-1β expression had significant higher GAGS score than absent-mild expression group (Table 4).

Discussion

This study shows that all patients with inflammatory acne vulgaris had dermal and epidermal IL-1β expression and the majority of the patients had perifollicular IL-1β expression. The level of IL-1β expression

| Table 2. Pathological results. |
|--------------------------------|
| All patients (N=20) |
| Epidermal hyperplasia, n (%) | Absent 2 (10) | Mild 11 (55) | Moderate 0 (0) | Severe 7 (35) |
| Follicular hyperkeratosis, n (%) | Absent 0 (0) | Mild 6 (30) | Moderate 9 (45) | Severe 5 (25) |
| Angiogenesis and the extent of dermal inflammatory response, n (%) | Absent 0 (0) | Mild 2 (10) | Moderate 11 (55) | Severe 7 (35) |
| Inflammatory infiltrate in epidermis, n (%) | Absent 14 (70) | Mild 1 (5) | Moderate 4 (20) | Severe 1 (5) |
| Inflammatory infiltrate in dermis, n (%) | Absent 0 (0) | Mild 3 (15) | Moderate 10 (50) | Severe 7 (35) |
| Dominant cell in epidermis, n (%) | Neutrophil 20 (100) |
| Dominant cells in dermis, n (%) | Monocyte 13 (65) | Monocyte and neutrophil 7 (35) |

| Table 3. Expression of IL-1β. |
|--------------------------------|
| All patients (N=20) |
| Dermal expression, n (%) | Absent 0 (0) | Mild 13 (65) | Moderate 6 (30) | Strong 1 (5) |
| Epidermal expression, n (%) | Absent 0 (0) | Mild 12 (60) | Moderate 8 (40) | Strong 0 (0) |
| Perifollicular expression, n (%) | Absent 2 (10) | Mild 14 (70) | Moderate 3 (15) | Strong 1 (5) |
| Type of cell in dermal expression, n (%) | Monocyte 20 (100) | Endothelial cell 3 (15) | Fibroblast 1 (5) | Giant cell 1 (5) |
| Type of cell in epidermal expression, n (%) | Basal cell 20 (100) | Squamous cell 20 (100) |
| Type of cell in perifollicular expression, n (%) | Monocyte 20 (100) |
Most of the samples in our study had epidermal hyperplasia. This is the primary abnormality found in acne lesions. The epithelium above the hair follicle is thick and increases adhesion between the keratinocytes. We noted that 10% did not have epidermal hyperplasia, possibly due to the small size of the biopsy sample and deviation around the hair follicle. All samples in this study had hyperkeratosis, which suggests that comedones were the initial lesions and then developed to other lesions such as nodules, inflammatory lesions, and scarring. Epidermal hyperplasia of the hair follicle led to the formation of comedones because it led to clogging of the hair follicle that kept keratin, sebum and bacteria. The exact cause of increased adhesion of keratinocytes and hyperkeratinosis is unknown; it is hypothesized that the factors that contribute to follicular hyperkeratosis are androgen stimulation, decreased linoleic acid, increased activity of IL-1 and the presence of macrophages around the sebaceous follicular unit and showed that P. acnes stimulated the secretion of IL-1β in acne lesions. Our study also showed the positive correlation of disease severity and the level of IL-1β expression in another study. IL-1β mRNA levels in pusules were 50-times higher than normal skin tissue. Some clinical reports suggest targeted therapy of IL-1β in patients with an autoinflammatory syndrome and acne vulgaris. These results suggest an association between clinical acne severity and IL-1β expression levels and thus, IL-1β may be a good target for new therapeutic agents.

There are several limitations in our study. First, the sample size was limited and there was no control group. This is because the resources were limited, and it was difficult to have a normal group as control regarding the ethical issue. Second, we could not evaluate deeper tissue as the disadvantage of punch biopsy. Third, the evaluation of IL-1β expression was semi-quantitative. It would be better if these were quantitative results.

**Conclusions**

In conclusion, this study demonstrates the increased immunoreactivity of IL-1β in the papule biopsies in patients with inflammatory acne vulgaris. Also, the levels of IHC IL-1β expression positively correlates with disease severity. IL-1β could be a good target for the development of new therapeutic agents. Future studies are required to confirm this finding and to develop new treatments for acne vulgaris.

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